

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49275 A3

(51) International Patent Classification⁷: **A61K 31/662**,
31/426, 31/40, A61P 29/00, 25/20, 25/22, 23/00, A61K
31/21, 31/24, 31/265, 31/425

**Khem [CA/CA]; 17 Jorene Drive, Kingston, Ontario K7M
3X5 (CA).**

(21) International Application Number: PCT/CA00/01523

(74) Agents: **SCRIBNER, Stephen, J. et al.**; Parteq Innova-
tions, Queen's University, Kingston, Ontario K7L 3N6
(CA).

(22) International Filing Date:
27 December 2000 (27.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/473,713 29 December 1999 (29.12.1999) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(63) Related by continuation (CON) or continuation-in-part
(CIP) to earlier application:
US 09/473,713 (CIP)
Filed on 29 December 1999 (29.12.1999)

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*):
QUEEN'S UNIVERSITY AT KINGSTON [CA/CA];
Kingston, Ontario K7L 3N6 (CA).

Published:
— with international search report

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **THATCHER, Gre-**
gory, R., J. [GB/CA]; 116 Toronto Street, Kingston, On-
tario K7L 4A7 (CA). **BENNETT, Brian, M.** [CA/CA]; 36
Fairway Hills Crescent, Kingston, Ontario K7M 2B4 (CA).
REYNOLDS, James, N. [CA/CA]; 52 Herchmer Cres-
cent, Kingston, Ontario K7M 2V9 (CA). **JHAMANDAS,**

(88) Date of publication of the international search report:
13 December 2001

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*



WO 01/49275 A3

(54) Title: METHODS AND COMPOSITIONS FOR MITIGATING PAIN USING NITRATE ESTERS

(57) Abstract: Methods and therapeutic compounds for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation in a subject are described. A subject is administered an effective amount of a therapeutic compound which is a nitrate ester. Novel pharmaceutical compositions are also described.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/01523

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/662 A61K31/426 A61K31/40 A61P29/00 A61P25/20
A61P25/22 A61P23/00 A61K31/21 A61K31/24 A61K31/265
A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, CANCERLIT, MEDLINE, SCISEARCH, EMBASE BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 693 676 A (GORFINE STEPHEN R) 2 December 1997 (1997-12-02)	1,25,35
Y	column 3, line 36 - line 49 column 4, line 14 - line 16 column 4, line 45 - column 5, line 13 claims 7-16 — — — — — — / —	1,27

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

5 September 2001

Date of mailing of the international search report

17.09.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Cielen, E

INTERNATIONAL SEARCH REPORT

Interr ial Application No
PCT/CA 00/01523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>XU JIMMY YU ET AL: "Activation of a NO-cyclic GMP system by NO donors potentiates beta-endorphin-induced antinociception in the mouse." PAIN, vol. 63, no. 3, 1995, pages 377-383, XP001004459 ISSN: 0304-3959 cited in the application abstract page 378, column 1, paragraph 2 page 381, column 2, paragraph 3 -page 382, column 2, paragraph 1 page 382, column 2, paragraph 3</p>	1,27
X	<p>WO 95 30641 A (NICOX LTD ;DEL SOLDATO PIERO (IT); SANNICOLO FRANCESCO (IT)) 16 November 1995 (1995-11-16) abstract page 3, paragraph 4 page 38, paragraph 4 -page 39, paragraph 1 examples 1A-1H page 55, paragraph 3 table 1</p>	1,25,35
X	<p>WO 98 25918 A (DROUX SERGE ;JOLY PASCAL (FR); PETIT FRANCIS (FR); GIGLIOTTI GIUSE) 18 June 1998 (1998-06-18) abstract page 1, line 5 - line 7 page 1, line 29 -page 2, line 15 page 7, line 36 -page 8, line 32 examples 1-5</p>	1,25,35
A	<p>WO 97 46521 A (UNIV KINGSTON ;BENNETT BRIAN M (CA); JHAMANDAS KHEM (CA); BOEGMAN) 11 December 1997 (1997-12-11) page 2, line 26 -page 5, line 7 page 8, line 39 - line 45 page 13, line 1 - line 18 page 16, line 9 - line 10 claims 1-8</p>	30,32-35

-/-

INTERNATIONAL SEARCH REPORT

Interr. ial Application No

PCT/CA 00/01523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>S. H. FERREIRA: "Inflammatory pain: the role of cytokines and its control by drugs which release nitric oxide." ANNALI DELL'ISTITUTO SUPERIORE DI SANITÀ, vol. 29, no. 3, 1993, pages 367-373, XP001004461 cited in the application page 368, column 1, paragraph 1 -column 2, paragraph 1 table 1 page 369, column 1, paragraph 5 page 371, column 1, paragraph 3 -column 2, paragraph 1</p>	1,27,29
X	<p>US 3 869 451 A (PHILLIPPS GORDON HANLEY ET AL) 4 March 1975 (1975-03-04)</p>	12
A	<p>column 1, line 7 - line 10 column 1, line 40 - line 48 column 3, line 28 - line 32 example 36 claim 1</p>	35
A	<p>YANG K ET AL: "SYNTHESIS OF NOVEL ORGANIC NITRATE ESTERS: GUANYLATE CYCLASE ACTIVATION AND TISSUE RELAXATION" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1, CHEMICAL SOCIETY. LETCHWORTH, GB, no. 11, 1996, pages 1073-1075, XP002039309 ISSN: 1472-7781 the whole document</p>	30,32-34
A	<p>EP 0 664 127 A (NIPPON ZOKI PHARMACEUTICAL CO) 26 July 1995 (1995-07-26) page 3, line 11 - line 44 page 5, line 14 - line 28</p>	12,28
A	<p>GOLDING P., MILLAR R., PAUL C., RICHARDS D.: "Nitration by oxides of nitrogen. Part 6. Preparation of di- and polynitrates by ring-opening nitration of epoxides by dinitrogen pentoxide (N2O5)" TETRAHEDRON , vol. 49, no. 32, 1993, pages 7037-7050, XP001016266 abstract page 7037, paragraph 1 page 7039, paragraph 1 - paragraph 4 page 7040; table 1 page 7047, paragraph 4</p>	32
	-/--	

INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/CA 00/01523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BURKE D H ET AL: "SODIUM NITROPRUSSIDE INDUCED HYPO THERMIA IN MICE" JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 66, no. 11, 1977, pages 1658-1660, XP001025601 EN ISSN: 0022-3549 abstract page 1658, column 2, paragraph 4 -page 1659, column 1, paragraph 1 page 1659, column 2, paragraph 5 -page 1660, column 1, paragraph 1	12-22
P,X	REYNOLDS J N ET AL: "Cognition enhancement and sedative-hypnotic properties with a novel nitrate ester." SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 26, no. 1-2, 2000, pages Abstract No.-653.9, XP001025586 30th Annual Meeting of the Society of Neuroscience;New Orleans, LA, USA; November 04-09, 2000 ISSN: 0190-5295	12,26
P,A	abstract	35
P,A	WO 00 54756 A (UNIV KINGSTON) 21 September 2000 (2000-09-21) page 4, line 3 - line 5 page 17, line 16 - line 30 page 19, line 4 - line 9 page 26, line 17 -page 34, line 15 page 36, line 18 - line 23 examples 1,2 claims 2,3,14,23,39,42	28,30, 32-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/01523

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT,
no additional fees are to be refunded.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

12-22, 24, 26, 28, 30-35
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-35 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed.

Moreover, present claims 27-30 relate to a method defined by reference to a desirable characteristic or property, namely "wherein the therapeutic compound modulates levels of the cyclic nucleotides cGMP and/or cAMP" or "wherein the therapeutic compound modulates guanylyl cyclase".

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search for the first, third, eighth and thirteenth inventions has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds which are supported by pharmacological or synthetic data, i.e. compounds IIIw and IIIx, and their use in the treatment of pain or in providing analgesia or sedation (inventions 1 and 3), compounds IIIaa and IIIaj, and their use in providing sedation (invention 8), compounds IVs, IVt, Ve, Vm, Vq, Vr, Vx, Vy, Vah, and their use in providing sedation (invention 13). The applicants attention is hereby drawn to the fact that the definition "organic radicals" in claims 1, 12 is considered to comprise hydrogen in view of the disclaimer having F1 is H, and in view of exemplified compounds.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11, 23, 25, 27, 29, 31, 32, 35 (partially)

A method for treating pain or providing analgesia in a subject, comprising administering a compound having the skeleton
 $\text{C1-CH}_2\text{-CH(ONO}_2\text{)-CH}_2\text{-P(O)(OCH}_2\text{CH}_3\text{)O-}$, i.e. compounds IIIw or IIIx, the compounds per se and pharmaceutical compositions containing them.

2. Claims: 1-11, 23, 25, 27, 29, 31, 32, 35 (partially)

A method for treating inflammation in a subject, comprising administering a compound having the skeleton
 $\text{C1-CH}_2\text{-CH(ONO}_2\text{)-CH}_2\text{-P(O)(OCH}_2\text{CH}_3\text{)O-}$, i.e. compounds IIIw or IIIx.

3. Claims: 12-22, 24, 26, 28, 30, 31, 32, 35 (partially)

A method for providing sedation in a subject, comprising administering a compound having the skeleton
 $\text{C1-CH}_2\text{-CH(ONO}_2\text{)-CH}_2\text{-P(O)(OCH}_2\text{CH}_3\text{)O-}$, i.e. compounds IIIw or IIIx.

4. Claims: 12-22, 24, 26, 28, 30, 31, 32, 35 (partially)

A method for mitigating anxiety in a subject, comprising administering a compound having the skeleton
 $\text{C1-CH}_2\text{-CH(ONO}_2\text{)-CH}_2\text{-P(O)(OCH}_2\text{CH}_3\text{)O-}$, i.e. compounds IIIw or IIIx.

5. Claims: 12-22, 24, 26, 28, 30, 31, 32, 35 (partially)

A method for providing anaesthesia in a subject, comprising administering a compound having the skeleton
 $\text{C1-CH}_2\text{-CH(ONO}_2\text{)-CH}_2\text{-P(O)(OCH}_2\text{CH}_3\text{)O-}$, i.e. compounds IIIw or IIIx.

6. Claims: 1-11, 23, 25, 27, 29, 31, 32, 35 (partially)

A method for treating pain or providing analgesia in a subject, comprising administering a compound having the skeleton
 $(\text{O}_2\text{NO})\text{CH}_2\text{-CH(ONO}_2\text{)-CH}_2\text{-CH}_2\text{-}$, i.e. compounds IIIaa or IIIaj, the compounds per se and pharmaceutical compositions containing them.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 1-11, 23, 25, 27, 29, 31, 32, 35 (partially)

A method for treating inflammation in a subject, comprising administering a compound having the skeleton
 $(O_2NO)CH_2-CH(ONO_2)-CH_2-CH_2-$, i.e. compounds IIIa or IIIaj.

8. Claims: 12-22, 24, 26, 28, 30, 31, 32, 35 (partially)

A method for providing sedation in a subject, comprising administering a compound having the skeleton
 $(O_2NO)CH_2-CH(ONO_2)-CH_2-CH_2-$, i.e. compounds IIIa or IIIaj.

9. Claims: 12-22, 24, 26, 28, 30, 31, 32, 35 (partially)

A method for mitigating anxiety in a subject, comprising administering a compound having the skeleton
 $(O_2NO)CH_2-CH(ONO_2)-CH_2-CH_2-$, i.e. compounds IIIa or IIIaj.

10. Claims: 12-22, 24, 26, 28, 30, 31, 32, 35 (partially)

A method for providing anaesthesia in a subject, comprising administering a compound having the skeleton
 $(O_2NO)CH_2-CH(ONO_2)-CH_2-CH_2-$, i.e. compounds IIIa or IIIaj.

11. Claims: 1-11, 23, 25, 27, 29, 31, 33-35 (partially)

A method for treating pain or providing analgesia in a subject, comprising administering a compound having the skeleton
 $(ONO_2)-CH_2-CH(ONO_2)-CH_2-S-$, i.e. compounds IVs, IVt, Ve, Vm, Vq, Vr, Vx, Vy, or Vah, the compounds per se and pharmaceutical compositions containing them.

12. Claims: 1-11, 23, 25, 27, 29, 31, 33-35 (partially)

A method for treating inflammation in a subject, comprising administering a compound having the skeleton
 $(ONO_2)-CH_2-CH(ONO_2)-CH_2-S-$, i.e. compounds IVs, IVt, Ve, Vm, Vq, Vr, Vx, Vy, or Vah.

13. Claims: 12-22, 24, 26, 28, 30, 31, 33-35 (partially)

A method for providing sedation in a subject, comprising administering a compound having the skeleton
 $(ONO_2)-CH_2-CH(ONO_2)-CH_2-S-$, i.e. compounds IVs, IVt, Ve, Vm, Vq, Vr, Vx, Vy, or Vah.

14. Claims: 12-22, 24, 26, 28, 30, 31, 33-35 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for mitigating anxiety in a subject, comprising administering a compound having the skeleton
(ON02)-CH₂-CH(ON02)-CH₂-S-, i.e. compounds IVs, IVt, Ve, Vm, Vq, Vr, Vx, Vy, or Vah.

15. Claims: 12-22, 24, 26, 28, 30, 31, 33-35 (partially)

A method for providing anaesthesia in a subject, comprising administering a compound having the skeleton
(ON02)-CH₂-CH(ON02)-CH₂-S-, i.e. compounds IVs, IVt, Ve, Vm, Vq, Vr, Vx, Vy, or Vah.

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern. Application No

PCT/CA 00/01523

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5693676 A	02-12-1997	US 5504117 A AT 196082 T AU 2282395 A CA 2168247 A CN 1131392 A DE 69518729 D DE 69518729 T DE 719145 T DK 719145 T EP 1051972 A EP 0719145 A ES 2092460 T GR 96300077 T IL 113448 A JP 9504037 T JP 2001026550 A PT 719145 T WO 9532715 A	02-04-1996 15-09-2000 21-12-1995 07-12-1995 18-09-1996 12-10-2000 31-05-2001 13-02-1997 02-01-2001 15-11-2000 03-07-1996 01-12-1996 31-01-1997 21-11-2000 22-04-1997 30-01-2001 31-01-2001 07-12-1995
WO 9530641 A	16-11-1995	IT 1269735 B IT 1274609 B AT 168986 T AT 184589 T AU 702662 B AU 2215695 A AU 678063 B AU 7809294 A BR 9407749 A BR 9507634 A CA 2173582 A CA 2190087 A DE 69412109 D DE 69412109 T DE 69512232 D DE 69512232 T DK 722434 T DK 759899 T WO 9509831 A EP 0722434 A EP 0759899 A ES 2120070 T ES 2139199 T GR 3032078 T HU 74446 A, B HU 75961 A JP 9503214 T JP 9512798 T RU 2136653 C RU 2145595 C SI 722434 T SI 759899 T US 5700947 A US 5861426 A US 5780495 A	15-04-1997 18-07-1997 15-08-1998 15-10-1999 25-02-1999 29-11-1995 15-05-1997 01-05-1995 12-02-1997 23-09-1997 13-04-1995 16-11-1995 03-09-1998 21-01-1999 21-10-1999 24-02-2000 16-11-1998 20-12-1999 13-04-1995 24-07-1996 05-03-1997 16-10-1998 01-02-2000 31-03-2000 30-12-1996 28-05-1997 31-03-1997 22-12-1997 10-09-1999 20-02-2000 31-12-1998 31-12-1999 23-12-1997 19-01-1999 14-07-1998
WO 9825918 A	18-06-1998	FR 2757159 A	19-06-1998
WO 9746521 A	11-12-1997	US 5807847 A	15-09-1998

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/CA 00/01523

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9746521 A		AU 725046 B AU 2883997 A BR 9709635 A EP 0915842 A JP 2000511197 T US 5883122 A	05-10-2000 05-01-1998 10-08-1999 19-05-1999 29-08-2000 16-03-1999
US 3869451 A	04-03-1975	GB 1376892 A AU 470344 B AU 3559171 A BE 775239 A CA 1010847 A CH 620226 A DE 2156094 A DK 136218 B FR 2113919 A IL 38124 A JP 56010320 B NL 7115502 A US 3952031 A ZA 7107587 A	11-12-1974 11-03-1976 17-05-1973 12-05-1972 24-05-1977 14-11-1980 18-05-1972 05-09-1977 30-06-1972 31-10-1977 06-03-1981 16-05-1972 20-04-1976 27-06-1973
EP 0664127 A	26-07-1995	JP 7196509 A US 5556862 A	01-08-1995 17-09-1996
WO 0054756 A	21-09-2000	AU 3267300 A	04-10-2000

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49275 A2

(51) International Patent Classification⁷: **A61K 31/00**

Khem [CA/CA]; 17 Jorene Drive, Kingston, Ontario K7M 3X5 (CA).

(21) International Application Number: PCT/CA00/01523

(22) International Filing Date:
27 December 2000 (27.12.2000)

(74) Agents: **SCRIBNER, Stephen, J. et al.**; Parteq Innovations, Queen's University, Kingston, Ontario K7L 3N6 (CA).

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/473,713 29 December 1999 (29.12.1999) US

(81) Designated States (*national*): AB, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/473,713 (CIP)
Filed on 29 December 1999 (29.12.1999)

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*):
QUEEN'S UNIVERSITY AT KINGSTON [CA/CA];
Kingston, Ontario K7L 3N6 (CA).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **THATCHER, Gregory, R., J.** [GB/CA]; 116 Toronto Street, Kingston, Ontario K7L 4A7 (CA). **BENNETT, Brian, M.** [CA/CA]; 36 Fairway Hills Crescent, Kingston, Ontario K7M 2B4 (CA). **REYNOLDS, James, N.** [CA/CA]; 52 Herchmer Crescent, Kingston, Ontario K7M 2V9 (CA). **JHAMANDAS,**

Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/49275 A2

(54) Title: METHODS AND COMPOSITIONS FOR MITIGATING PAIN

(57) Abstract: Methods and therapeutic compounds for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation in a subject are described. A subject is administered an effective amount of a therapeutic compound which is a nitrate ester. Novel pharmaceutical compositions are also described.

METHODS AND COMPOSITIONS FOR MITIGATING PAIN

FIELD OF THE INVENTION

This invention relates to nitrate esters and use thereof in mitigating pain and
5 effecting analgesia. More particularly this invention relates to organic nitrates which have
therapeutic utility as analgesics, anti-inflammatory agents and sedatives.

BACKGROUND OF THE INVENTION

The nitrate ester, glyceryl trinitrate (GTN), or nitroglycerin, has been used as a
10 vasodilator in the treatment of angina pectoris for over a hundred years, and the dominant,
contemporary belief is that GTN exerts its therapeutic effect through *in vivo* release of
nitric oxide (NO). Other organic nitrates (nitrate esters), such as isosorbide dinitrate, have
also been identified as effective and clinically important vasodilators. NO itself has been
identified as Endothelium Derived Relaxing Factor (EDRF) and several classes of
15 compounds, for example nitrosothiols, in addition to organic nitrates, have been proposed
as NO donors or NO prodrugs.

Several organic nitrates, in which an alkyl mononitrate is appended to a moiety
with analgesic properties, such as aspirin (ASA) or a Non-Steroidal Anti-Inflammatory
Drug (NSAID) have been reported as analgesics which possess reduced gastro-intestinal
20 irritation and ulceration properties, purportedly through release of NO. The
combination of the vasodilator nitroglycerin with opioid analgesics such as morphine, has
been suggested to be effective in the management of both surgical and cancer pain.
However, no attempt has been made to develop organic nitrates themselves as analgesic
agents, that is, organic nitrates that do not rely on an ASA or NSAID moiety, nor an
25 opiate, for analgesic properties. Thus, there is a need for synthetic organic nitrates as new
and useful therapeutic agents for treatment and mitigation of pain associated with disease
states and chemotherapy of those disease states.

OBJECT OF THE INVENTION

It is an object of the present invention to provide methods and compositions for use in treating pain and/or conditions associated with pain. Another object of the present invention is to provide methods and compositions for providing analgesia and/or sedation.

5

BRIEF DESCRIPTION OF THE INVENTION

The present invention is based, at least in part, on the recognition that, although the potent vasodilatory effects of organic nitrates may be either (a) deleterious to or, alternatively, (b) synergistic with their analgesic effects, *regulation* of these two effects is required for the development of therapeutic agents useful in treatment and mitigation of pain. Pain may be treated or mitigated by, for example, an analgesic, anti-inflammatory and/or sedative agent.

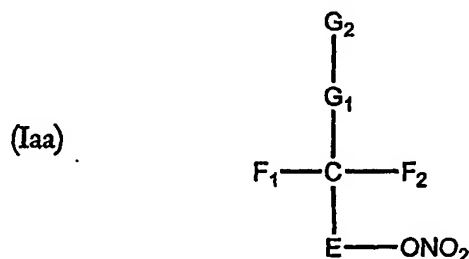
Possible deleterious effects of organic nitrates may arise, for example, through an NO-donor potentiating hyperalgesia via a cyclic guanosine-3',5'-monophosphate (cGMP)-independent mechanism. Alternatively, synergistic effects of organic nitrates may arise, for example, through the ability of an NO-donor to induce analgesia by activation of soluble guanylyl cyclase (GCase) and elevation of cGMP levels. The present invention relates to methods for treating or mitigating pain through use of an organic nitrate, wherein regulation of these two effects is achieved. According to the invention, selection of an appropriate organic nitrate provides modulation and balance between the ability of the organic nitrate to release NO and its potency for GCase activation. Inasmuch as gastrointestinal toxicity is known to be a deleterious side effect of some analgesic drugs and that NO donor molecules are gastro-protective, it is set forth herein that therapeutic analgesia can be achieved through utilization of an appropriate organic nitrate. This statement is based, at least in part, on bioassay data on such compounds.

This invention provides methods and compositions which are useful in treating pain, inhibiting inflammation, and/or providing analgesia. Methods of the invention involve administering to a subject a therapeutic compound (nitrate ester) which provides analgesia. The methods and compositions of the invention are useful for the treatment and mitigation of pain associated with disorders and disease states and chemotherapy of those

30

disease states. The methods and compositions of the invention can be used therapeutically to treat acute, chronic and/or inflammatory pain in conditions such as, but not limited to, nerve injury, post-herpetic neuralgia, arthritis, diabetic neuropathy, dysmenorrhea, endometriosis, phantom limb pain, pain associated with cancer and post-operative pain, or
 5 can be used prophylactically in a subject susceptible or predisposed to these conditions. In certain preferred embodiments, a therapeutic compound used in the method of the invention interacts with guanylyl cyclase, effecting analgesia. In other preferred embodiments, a therapeutic compound used in the method of the invention modulates levels of the cyclic nucleotides cyclic guanosine-3',5'- monophosphate (cGMP) and cyclic
 10 adenosine-3',5'- monophosphate (cAMP).

In one aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic
 15 compound is of the formula (Iaa):



in which E, F₁, F₂, G₁, and G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

25 with the proviso that when E and G₁ are methylene groups and F₁ is H, G₂ is not a nitrate group, nor R^N-Z^N;

wherein R^N is any aryl or heteroaryl group and Z^N is (CO)_{mm}-X^N_{nn}-Y^N_{oo};

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

In a preferred embodiment, F_2 is a nitrate group and E , F_1 , G_1 , G_2 are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

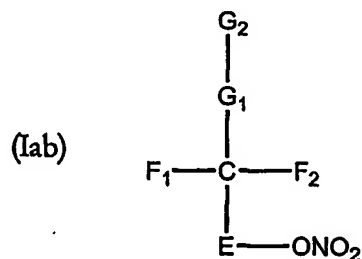
with the proviso that when E and G_1 are methylene groups and F_1 is H, G_2 is not a
5 nitrate group, nor R^N-Z^N ;

wherein R^N is any aryl or heteroaryl group and Z^N is $(CO)_{mm}-X_{nn}^N-Y_{oo}^N$;

wherein mm , nn , oo are 0 or 1 and X^N, Y^N are NH , NR^{NN} , O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

In another aspect, the invention provides a method for treating pain, treating or
10 inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety
and/or providing anaesthesia in a subject, comprising administering to a subject in need
thereof an effective amount of a therapeutic compound, wherein the therapeutic
compound is of the formula (Iab):



in which E , F_1 , F_2 , G_1 , and G_2 are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

with the proviso that when CF_1F_2 and G_1G_2 are not substituted or unsubstituted
25 counterions;

wherein E is C_1 alkane, CF_1F_2 and G_1G_2 are not both C_1 to C_3 alkyl radicals bearing one or more nitrate groups or an O linkage.

In a preferred embodiment, F_2 is a nitrate group and E , F_1 , G_1 , G_2 are the same or different organic radicals which may be joined in cyclic ring systems, and which may
30 contain inorganic counterions;

with the proviso that when E and G₁ are methylene groups and F₁ is H, G₂ is not a nitrate group, nor R^N-Z^N;

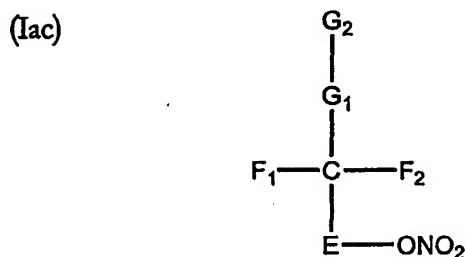
wherein R^N is any aryl or heteroaryl group and Z^N is (CO)_{mm}-X^N_{nn}-Y^N_{oo};

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

5 wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

In another aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic

10 compound is of the formula (Iac):



15 in which E may or may not exist, and E, F₁, F₂, G₁, and G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

20 with the proviso that when E does not exist, F₁, F₂, and G₁ are H, methylene, C₁-C₁₂ alkyl, aryl, or alkylaryl groups, G₂ is not R^N-Z^N;

wherein R^N is any aryl or heteroaryl group and Z^N is (CO)_{mm}-X^N_{nn}-Y^N_{oo};

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

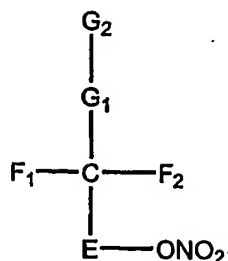
25 wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

In another aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic

30 compound is of the formula (Ib):

6

(Ib)



in which F_2 is an organic radical which may be joined in a cyclic ring system with G_2 , and which may contain inorganic counterions; E and G_1 are both methylene groups; F_1 is H; and G_2 is R^N-Z^N ;

wherein R^N is an organic radical possessing a heteroaryl group containing P or S atoms where said P or S are positioned β , γ , or δ to a nitrate group as identified in formula I; and Z^N is $W^N_{mm}-X^N_{nn}-Y^N_{oo}$;

wherein mm, nn and oo are 0 or 1; and W^N , X^N , Y^N are NH, NR^{NN} , CO, O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

In a preferred embodiment, F_2 is a nitrate group; E and G_1 are methylene groups; F_1 is H; and G_2 is R^N-Z^N ;

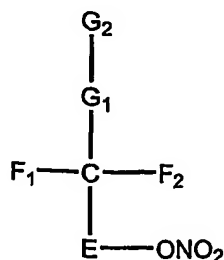
wherein R^N is an organic radical possessing a substituted or unsubstituted heteroaryl group containing P or S atoms where said P or S are positioned β , γ , or δ to a nitrate group as identified in formula I; and Z^N is $W^N_{mm}-X^N_{nn}-Y^N_{oo}$;

wherein mm, nn, oo are 0 or 1 and W^N , X^N , Y^N are NH, NR^{NN} , CO, O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

In another aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ic):

(Ic)



in which E is $(R^1R^2C)_m$ and $G_2-G_1-CF_1F_2-$ is $R^{19}-(R^3R^4C)_p-(R^{17}R^{18}C)_n-$;

wherein: m, n, p are integers from 0 to 10;

$R^{3,17}$ are each independently hydrogen, a nitrate group, or A; and

$R^{1,4}$ are each independently hydrogen, or A;

- 5 where A is selected from a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring,
10 which optionally may contain O, S, NR^6 and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between R^1 and R^3 and/or between R^{17} and R^4 , which optionally may contain O, S, NR^6 and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or
15 heterocyclic groups; a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain) containing carbonyl linkages (e.g., $C=O$, $C=S$, $C=NOH$), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a substituted
20 or unsubstituted heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties); arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy;

- wherein X is F, Br, Cl, NO_2 , CH_2 , CF_2 , O, NH, NMe, CN, $NHOH$, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$,
25 $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_2HM , PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)$, $C(O)R^{12}$, $C(O)(OR^{13})$, PO_2H , PO_2M , $P(O)(OR^{14})$, $P(O)(R^{15})$, SO, SO_2 , $C(O)(SR^{13})$, SR^5 , SSR^7 or SSR^5 ;

- Y is F, Br, Cl, CH_3 , CF_2H , CF_3 , OH, NH_2 , NHR^6 , NR^6R^7 , CN, $NHOH$, N_2H_3 ,
30 $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_2HM ,

PO_3M_2 , $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$, $\text{P}(\text{O})(\text{OR}^{16})(\text{OM})$, $\text{P}(\text{O})(\text{R}^{15})(\text{OR}^8)$, $\text{P}(\text{O})(\text{OM})\text{R}^{15}$, CO_2M ,
 CO_2H , CO_2R^{11} , $\text{C}(\text{O})\text{R}^{12}$, $\text{C}(\text{O})(\text{OR}^{13})$, $\text{C}(\text{O})(\text{SR}^{13})$, SR^5 , SSR^7 or SSR^5 , or does not exist;

R^2 , R^5 , R^{18} , R^{19} are optionally hydrogen, A or X-Y;

R^6 , R^7 , R^8 , R^9 , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} are the same or different alkyl or acyl
 5 groups containing 1-24 carbon atoms which may contain 1-4 ONO_2 substituents; or $\text{C}_1 - \text{C}_6$ connections to $\text{R}^1 - \text{R}^4$ in cyclic derivatives which may contain 1-4 ONO_2 substituents; or are each independently hydrogen, a nitrate group, or A;

M is H, Na^+ , K^+ , NH_4^+ , $\text{N}^+\text{H}_k\text{R}^{11}_{(4-k)}$ where k is 0-3; or other pharmaceutically acceptable counterion;

10 and with the proviso that when $m = n = p = 1$ and R^{19} , R^2 , R^{18} , $\text{R}^1 = \text{H}$ and R^{17} , R^3 are nitrate groups, R^4 is not H.

In one embodiment, R^{19} is X-Y.

In other embodiments, E, F_1 , F_2 , G_1 , and G_2 are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic
 15 counterions;

wherein when $m = 0$, $n = 1$, $p = 1$, R^{17} , R^{18} , R^3 , R^4 are H, $\text{C}_1 - \text{C}_{12}$ alkyl, aryl, or alkylaryl groups, and R^{19} is not $\text{R}^N - \text{Z}^N$;

wherein $\text{R}^N - \text{Z}^N$ is a substituted or unsubstituted aryl group or a substituted or unsubstituted heterocyclic group;

20 wherein R^N is any aryl or heteroaryl group and Z^N is $(\text{CO})_{mm} - \text{X}^N_{nn} - \text{Y}^N_{oo}$;

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN} , O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($\text{C}_1 - \text{C}_{12}$).

In other embodiments, R^1 and R^3 are the same or different and selected from H and $\text{C}_1 - \text{C}_4$ alkyl chains, which chains may include one O linking R^1 and R^3 to form pentosyl,
 25 hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;

R^2 and R^4 are the same or different and selected from H, a nitrate group, $\text{C}_1 - \text{C}_4$ alkyl chains optionally bearing 1-3 nitrate groups, and acyl groups $(-\text{C}(\text{O})\text{R}^5)$;

R^7 , R^{11} are the same or different $\text{C}_1 - \text{C}_8$ alkyl or acyl;

$R^5, R^6, R^8, R^9, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$ are the same or different and are alkyl groups containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents; or C_1 or C_2 connections to $R^1 - R^3$ in cyclic derivatives; and

M is H, Na^+ , K^+ , NH_4^+ or $N^+H_kR^{11}_{(4-k)}$, where k is 0-3.

5 In other embodiments, $m = 1, n = 1, p = 1$.

In further embodiments, X is $CH_2, O, NH, NMe, CN, NHOH, N_2H_3, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, S, SCN, SCN_2H_2(R^{15})_2, SCN_2H_3(R^{15}), SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SH, SR^7, SO_2M, S(O)R^8, S(O)_2R^9, S(O)OR^8, S(O)_2OR^9, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H,$
 10 $CO_2R^{11}, C(O), C(O)R^{12}, C(O)(OR^{13}), PO_2M, P(O)(OR^{14}), P(O)(R^{13}), SO, SO_2, C(O)(SR^{13}),$ or SSR^5 ; and

Y is $CN, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, SCN, SCN_2H_2(R^{15})_2, SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SR^7, SO_2M, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O)R^{12}, C(O)(SR^{13}), SR^5,$ or SSR^5 ,
 15 or does not exist.

In yet further embodiments, $R^5, R^6, R^8, R^9, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$ are the same or different and are alkyls containing 1-12 carbon atoms; or C_1 or C_2 connections to R^1 or R^3 in cyclic derivatives;

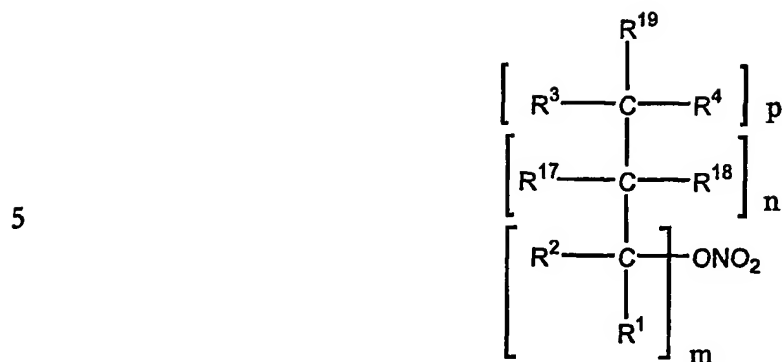
X is $CH_2, O, NH, NMe, S, SO_3M, SH, SR^7, SO_2M, S(O)R^8, S(O)_2R^9, S(O)OR^8, S(O)_2OR^9, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), PO_3HM$ or $P(O)(OM)R^{15}$; and
 20

Y is $SO_2M, SO_3M, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), SR^5, SR^7$ or SSR^5 , or does not exist.

In the above aspects and embodiments, R^{NN} is preferably a short chain alkyl group ($C_1 - C_8$).
 25

In preferred embodiments, therapeutic compounds of the invention act as analgesic, sedative and/or anti-inflammatory agents. Preferred therapeutic compounds for use in the invention include compounds having the formula (Formula II):

30 (Formula II)



in which: m and n and p are integers from 0 to 10;

10 $\text{R}^{3,17}$ are each independently hydrogen; a nitrate group; or A;

$\text{R}^{1,4}$ are each independently hydrogen; or A;

where A is selected from: a substituted or unsubstituted aliphatic group (preferably a branched, or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S, NR^6 and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between R^1 and R^3 and/or between R^{17} and R^4 , which optionally may contain O, S, NR^6 and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), containing carbonyl linkages (e.g., $\text{C}=\text{O}$, $\text{C}=\text{S}$, $\text{C}=\text{NOH}$), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a substituted or unsubstituted heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy.

30 R^2 , R^5 , R^{18} , R^{19} are optionally hydrogen; or A; or X-Y;

where X is F, Br, Cl, NO₂, CH₂, CF₂, O, NH, NMe, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₂HM, PO₃HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵,
 5 CO₂M, CO₂H, CO₂R¹¹, C(O), C(O)R¹², C(O)(OR¹³), PO₂H, PO₂M, P(O)(OR¹⁴), P(O)(R¹³), SO, SO₂, C(O)(SR¹³), SR⁵, SSR⁷ or SSR⁵,

Y is F, Br, Cl, CH₃, CF₂H, CF₃, OH, NH₂, NHR⁶, NR⁶R⁷, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₂HM,
 10 PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O)R¹², C(O)(OR¹³), C(O)(SR¹³), SR⁵, SSR⁷ or SSR⁵, or does not exist;

R⁶, R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶ are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO₂ substituents; or C₁-C₆ connections to R¹ - R⁴ in cyclic derivatives; or are each independently hydrogen; a
 15 nitrate group; or W;

M is H, Na⁺, K⁺, NH₄⁺, N⁺H_kR¹¹_(4-k) where k is 0-3, or other pharmaceutically acceptable counterion;

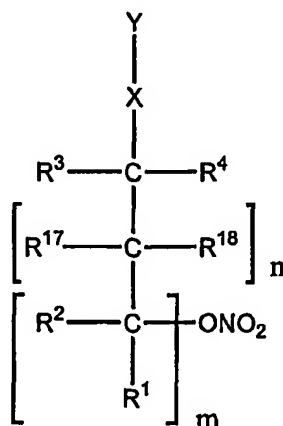
and with the proviso that, when m = n = p = 1; R¹⁹, R², R¹⁸, R¹ = H; R¹⁷, R³ are nitrate groups; that R⁴ is not H or C₁ - C₃ alkyl.

20 In certain preferred embodiments, therapeutic compounds of the invention are analgesic, sedative and/or anti-inflammatory agents. Preferred therapeutic compounds for use in the invention include compounds in which R¹⁹ is X-Y. In a particularly preferred embodiment: R¹⁹ is X-Y and R⁵, R⁶, R⁸, R⁹, R¹⁰, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶ are the same or different alkyl groups containing 1-24 carbon atoms which may contain 1-4 ONO₂
 25 substituents, or C₁ or C₂ connections to R¹ - R³ in cyclic derivatives; R¹ and R³ are the same or different and selected from H, C₁-C₄ alkyl chains, which may include one O, linking R¹ and R³ to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents; R² and R⁴, are the same or different and selected from H, a nitrate group, C₁-C₄ alkyl optionally bearing 1-3 nitrate groups, and acyl
 30 groups (-C(O)R⁹); R⁷, R¹¹ are the same or different C₁ - C₈, alkyl or acyl.

In certain embodiments in which R_{19} is X-Y, $m, p = 1$, and $n = 0$. In other embodiments in which R_{19} is X-Y, X is selected from CH_2 , O, NH, NMe, CN, NHOH, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)$, $C(O)R^{12}$, $C(O)(OR^{13})$, PO_2M , $P(O)(OR^{14})$, $P(O)(R^{13})$, SO, SO_2 , $C(O)(SR^{13})$, SSR^4 . In another embodiment in which R_{19} is X-Y, Y is selected from CN, $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , SCN, $SCN_2H_2(R^{15})_2$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SR^4 , SO_2M , PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)R^{12}$, $C(O)(SR^{13})$, SR^5 , SSR^5 , or does not exist.

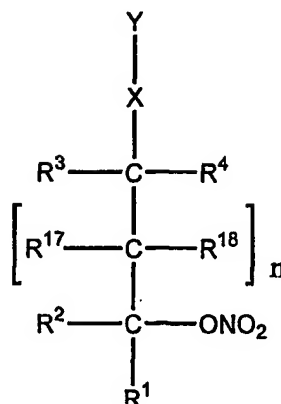
In a further embodiment, X and/or Y contain a sulfur-containing functional group. In certain preferred embodiments, the compound of the invention comprises a heterocyclic functionality, more preferably, a nucleoside or nucleobase. In further preferred embodiments, the compound of the invention comprises a carbocyclic functionality, more preferably, a steroidal or carbohydrate moiety.

In another aspect, a therapeutic compound of the invention is represented by the formula (Formula III):



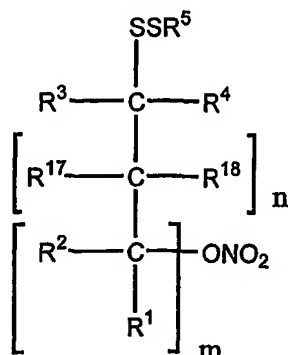
in which: m is 1-10; R^{1-18} , X, and Y have the meaning as defined above. In certain preferred embodiments, $R^6 - R^{16}$ are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO_2 substituents, or $C_1 - C_6$ connections to $R^1 - R^4$ in cyclic derivatives. In certain preferred embodiments, R^{18} is A and $n = 1$.

In preferred embodiments, a therapeutic compound of the invention is represented by the formula (Formula IV):



- in which: $R^3, R^1 = H$; $n, R^2 R^{4-18}$, X , and Y have the meaning as defined above. In preferred embodiments, X is CH_2 or does not exist, and Y is selected from, F, Br, Cl, CH_3 , CF_2H , CF_3 , OH, NH_2 , NHR^6 , NR^6R^7 , CN, $NHOH$, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_2HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)R^{12}$, $C(O)(OR^{13})$, $C(O)(SR^{13})$, SR^5 , SSR^7 or SSR^5 . In certain preferred embodiments, R^2 and R^4 are optionally H, a nitrate group or a connection to $R^{15}-R^{16}$ in cyclic derivatives.

In certain preferred embodiments, a compound of the invention is represented by the formula (Formula V):



- in which m, n, R^{1-18} , X , and Y have the meaning as defined above.

In another aspect, the invention includes novel compounds useful for treating pain, mitigating inflammation, effecting analgesia and/or providing sedation. The compounds of the invention can be represented by the structures shown hereinbelow, for example, the structures of Formula III, IV and V. Novel compounds of the invention include nitrates
5 IIIr – IIIaj, IVn – Ivt, and Vd – Vba, whose syntheses are described in the following examples.

The invention also provides methods for treating a disease state associated with inflammation, comprising administering to a subject an effective amount of a therapeutic compound having a formula set forth above, such that a disease state associated with
10 inflammation is treated.

The invention further provides methods for treating a disease state or disorder in which a level of sedation is desired, comprising administering to a subject an effective amount of a sedative therapeutic compound having a formula set forth above, such that a disease state or disorder is treated.

15 The invention provides methods for effecting analgesia comprising administering to a subject an effective amount of a therapeutic compound having a formula set forth above, such that analgesia is effected.

The invention further provides novel pharmaceutical compositions for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation. A said
20 pharmaceutical composition comprises a therapeutic compound of the invention in an effective amount for the particular indication and a pharmaceutically acceptable vehicle.

The invention also provides packaged pharmaceutical compositions for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation. The packaged pharmaceutical compositions include a therapeutic compound of the invention and
25 instructions for using the pharmaceutical composition for treatment of inflammation and/or pain.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the effect of IVd neat (diamonds); with added L-
30 cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble

GCase activity in rat aorta homogenate normalized to the maximal GTN response. Bars represent the mean \pm standard errors calculated separately for each point.

Figure 2 is a graph showing the comparison of GTN (squares), III_m (circles) and IV_h (triangles) with added L-cysteine (1 mM) on soluble GCase activity in rat aorta homogenate (a), and rat hippocampus homogenate (b). Data points represent the mean of duplicate determinations carried out in identical GCase preparations.

Figure 3 is a graph showing the comparison of GTN (squares), V_a (circles) and V_b (triangles) with added L-cysteine (1 mM) on soluble GCase activity in rat aorta homogenate (a), and rat hippocampus homogenate (b). Data points represent the mean \pm standard errors calculated separately for each point (n=8-11).

Figure 4 is a graph showing the comparison of cyclic GMP accumulation in isolated rat aorta induced by diluent (basal, open bar), GTN (filled bar), V_a (stippled bar), or III_m (hatched bar). Segments of rat aorta were exposed to diluent, 1 μ M drug (a), or 10 μ M drug (b) for 1 min and cyclic GMP content determined by radioimmunoassay. Data are the mean \pm standard errors (a, n=8; b, n=5).

Figure 5 is a graph showing the comparison of cyclic GMP accumulation in isolated rat aorta induced by diluent (basal, open bar), GTN (filled bar), IV_k (stippled bar), V_b (cross-hatched bar), or V_c (hatched bar). Segments of rat aorta were exposed to diluent, 1 μ M drug (a), or 10 μ M drug (b) for 1 min and cyclic GMP content determined by radioimmunoassay. Data are the mean \pm standard errors (a, n=5; b, n=4).

Figure 6 is a graph showing cyclic GMP accumulation in rat hippocampal slices induced by diluent (basal, open bar), GTN (filled bar), and V_a (stippled bar). Sections of rat hippocampus (400 μ m) were prepared and exposed to diluent, 10 μ M drug (a) or 100 μ M drug (b) for 3 min and cyclic GMP content determined by radioimmunoassay. Data are the mean \pm standard errors (a, n=4; b, n=5).

Figure 7 is a graph showing the comparison of relaxation of isolated rat aorta induced by GTN (squares), V_a (open triangles), compound IV_c (diamonds), compound IV_d (open squares), compound IV_f (triangles), and compound IV_g (open diamonds). Data points represent the mean \pm standard errors (n=5-8).

Figure 8 is a graph showing the comparison of relaxation of isolated rat aorta induced by GTN (squares), IVk (open triangles), Vb (diamonds), IIIIm (open squares), Vc (triangles), and IVh (open diamonds). Data points represent the mean \pm standard errors (n=3-8).

5 Figure 9 is a graph showing the comparison of the percent change in mean arterial pressure (MAP) in conscious unrestrained rats after subcutaneous administration of 400 μ mol/kg GTN (squares) or Va (open circles). Data points represent the mean \pm standard errors (n=6).

10 Figure 10 is a graph showing the comparison of the percent change in mean arterial pressure in Inactin anaesthetized rats after intravenous bolus injection of GTN (squares) or Va (open circles). Data points represent the mean \pm standard errors (n=4).

15 Figure 11 is a graph showing the plasma levels (μ M) of Vb (circles) and its mononitrate metabolite Vc (open squares) after subcutaneous administration of 200 μ mol/kg Vb in conscious unrestrained rats. Data points represent the mean of two experiments.

Figure 12 is a graph showing the relaxation induced by compound IVd (a) and IVc (b) in untreated (squares) and GTN-tolerant (circles) isolated rat aorta. Aortae were made tolerant by treatment with 0.5 mM GTN for 30 min. Data points represent the mean \pm standard deviation (n= 3-6).

20 Figure 13 is a graph showing the effect of Vm and Va in the mouse writhing test. Vm produced a dose-dependent analgesic effect (A). Va also produced analgesia in the mouse writhing test (B). Data are mean \pm standard errors (n = 10-20).

25 Figure 14 is a graph showing the effect of Vm on paw flinches in rats after injection of formalin into the footpad. In comparison to vehicle-treated control animals, Vm decreased the initial pain response to formalin injection (at time = 0, *, p < 0.05), and the secondary hyperalgesia that developed between 20 and 40 minutes after formalin injection (A). For each animal, a cumulative score (total number of flinches over 60 minutes) was calculated (B). Vm significantly decreased cumulative paw flinches for 60 minutes after formalin injection. Data are mean \pm standard errors (N = 6-7).

Figure 15 is a graph showing (A) the effects of chlormethiazole (CHLOR) and IVk (200 μ M of each) on the membrane current induced by 10 μ M GABA in a *Xenopus* oocyte expressing the $\alpha 1\beta 1\gamma 2$ L isoform of human recombinant GABA_A receptors, and (B) the effect of IVk on loss of the righting reflex in mice after intraperitoneal injection of 100 mg/kg and 200 mg/kg. Data in part B are mean \pm standard error for three animals at each dose.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention there are provided methods and compositions useful in the treatment of pain. Methods of the invention involve administering to a subject an effective amount of a therapeutic compound which provides analgesia, mitigates inflammation and/or provides sedation. In some embodiments, the invention provides prophylactic methods for avoiding or pre-empting pain, inflammation and the like in a subject. For example, the subject may be susceptible or predisposed to these conditions, e.g., arthritic. Alternatively, the subject may be undergoing a course of treatment, e.g. cancer chemotherapy, which produces pain, inflammation or the like as a side effect. Methods of the invention may be practiced prior to, concurrently with, or after such a course of treatment.

In accordance with the invention, analgesic, anti-inflammatory and/or sedative activity can be effected by modulating an interaction with guanylyl cyclase (GCase; the enzyme responsible for cGMP production in various areas of the body), and/or by modulating levels of cGMP and cAMP messenger molecules.

As used herein, the term "treating" pain encompasses preventing, ameliorating, mitigating and/or managing pain and/or conditions that may cause pain, such as inflammation. As used herein, "inhibiting" pain or inflammation encompasses preventing, reducing and halting progression of same. The terms "organic nitrate" and "nitrate ester" are used interchangeably herein, with no distinction drawn between them.

According to one aspect of the invention, there is provided a method for treating pain in a subject, comprising administering to the subject an effective amount of a compound (nitrate ester) which effects analgesia in the subject. Preferably, analgesia is

effected by stimulating GCase. *In vivo*, GCase activation is effected by nitric oxide (NO), the proximal activator of GCase, which is generated endogenously by enzyme action on arginine in response to many biological triggers (J. R. Stone and M. A. Marletta, *Biochemistry* (1996) 35, 1093). One of the major targets for organic nitrates is GCase
5 activation, resulting in the production of cGMP. In this respect, organic nitrates act as NO-surrogates. In some cases, there is evidence that organic nitrates may act also as NO-donors, but these two properties should be differentiated and can be modulated by choice of the appropriate organic nitrate. That is, NO-donors release NO which interacts with specific biological targets. In contrast, NO-surrogates interact with the same biological
10 targets, but such interactions are not mediated by prior NO release from the NO-surrogate. In further contrast, NO-mimetics mediate the same physiological events as NO, regardless of the specific target.

Experimental evidence obtained in a number of *in vivo* model systems supports the notion that elevated levels of cGMP help effect analgesia. Sodium nitroprusside (SNP),
15 which releases NO non-enzymatically, blocked the hyperalgesic effect of prostaglandin (PGE₂) in a rat paw pressure test (Ferreira *et al.*, 1991). Moreover, this effect was potentiated by an inhibitor of cGMP phosphodiesterase, and was blocked by an inhibitor of GCase (Ferreira *et al.*, 1991). The peripheral analgesic effects of morphine were attributed to elevations of cGMP levels in sensory nerve fibres (Ferreira *et al.*, 1991;
20 Granados-Soto *et al.*, 1997) in both the rat paw pressure and formalin tests, since inhibition of GCase activity attenuated the analgesic effects of locally applied morphine. Activation of the NO-cGMP system by NO donors such as SNP has also been reported to potentiate beta-endorphin-induced analgesia in thermal tail-flick test in mice (Xu *et al.*, 1995), an effect that was potentiated by a selective inhibitor (zaprinast) of a cGMP-specific
25 phosphodiesterase.

Sensitization of sensory nerve fibres leading to hyperalgesia is assumed to involve increased concentrations of cAMP and calcium ions in sensory neurons, a process that may be attenuated or counteracted by activation of the NO-cGMP pathway (Ferreira, 1993; Cunha *et al.*, 1999).

30 In accordance with another embodiment of the invention, administration of an effective amount of a therapeutic compound to a subject effects analgesia in the subject by

modulating levels of cAMP and/or cGMP. For example, it has been shown that NO-donors modulate hyperalgesia via modulation of levels of cAMP, separately from and in addition to modulation of cGMP levels, in rat models of pain and nociceptor sensitization (Aley *et al.* 1998). Thus, modulation of cAMP/cGMP levels is expected to be effective in
5 inducing analgesia and in pain management in individuals suffering injury, disease or aging.

In a further embodiment of the invention, there is provided a method for treating or inhibiting inflammation in a subject, comprising administering to the subject an effective amount of a compound which mitigates inflammation in the subject. Preferably, inflammation is mitigated by modulation of levels of cGMP/cAMP. Inflammatory
10 hyperalgesia has been shown to be linked directly to the NO-cGMP pathway (Ferreira, 1993).

We have shown in our co-pending application USSN 09/267,379, filed March 15, 1999, which is hereby incorporated by reference, that novel nitrate esters have differential effects to activate soluble GCase and to cause cGMP accumulation in vascular and brain
15 tissue. Further, we have shown that the structure of the organic nitrate can be varied to alter potency and efficacy towards both activation of GCase and accumulation of cGMP and effects resulting from these processes in intact tissue, such as aortic strip relaxation. Activation of GCase and accumulation of cGMP have been shown to be important in the induction of analgesia. We show herein that novel organic nitrates are effective analgesics
20 in animal models of pain management. The mouse writhing model, with a relatively short time course of minutes, is a preclinical model of acute pain; whereas formalin injection in the rat paw is a preclinical model of acute and sensitization pain with a time course of minutes to hours, which effectively mimics the hyperalgesia/allodynia underlying pain due to tissue damage (Yaksh, 1999).

25 In a further embodiment, the invention relates to a method for providing sedation and/or anaesthesia in a subject, comprising administering to the subject an effective amount of a compound (organic nitrate) which effects sedation or anaesthesia in the subject. In certain aspects, the invention provides methods and compositions useful for reducing anxiety, and/or aiding or inducing sleep.

30 γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian (including human) central nervous system. GABA acts on three major classes

of neurotransmitter receptor, designated type A (GABA_A), type B (GABA_B) and type C (GABA_C). GABA_A receptors play an important role in regulating many behavioural and physiological functions. Thus, drugs that modulate GABA_A receptor function are among the most widely used in clinical medicine. For example, drugs that selectively potentiate

5 GABA_A receptor function (such as the benzodiazepines) are extensively used to relieve anxiety, produce sedation and induce sleep. Given the importance of this receptor mechanism in clinical medicine, there is a constant search for new chemical entities that modulate GABA_A function. Adverse effects that are associated with currently available hypnotic agents include residual sedation and psychomotor impairment; anterograde

10 amnesia and cognitive impairment; daytime anxiety; rebound insomnia; and drug tolerance and physical dependence. Many of these adverse effects, particularly cognitive and psychomotor impairment, occur with greater frequency in the elderly, for whom these drugs are often prescribed. We show herein that organic nitrates that act as positive allosteric modulators of GABA_A receptor function have sedative properties in the whole

15 animal that are comparable to known drugs. This effect of organic nitrates has not previously been recognized or reported. Our findings provide direct evidence that nitrate esters are useful as sedative agents. Such agents are useful as therapeutics for treating conditions such as, for example, anxiety and pain associated with disease states; and as hypnotic agents. Moreover, we report herein that organic nitrates that possess sedative

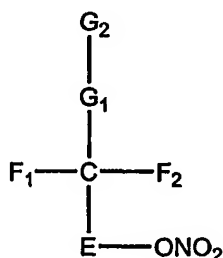
20 properties can also act as cognition enhancing agents, and therefore are expected to have a significantly decreased incidence of residual cognitive and motor impairment compared to currently available sedative-hypnotic compounds. According to the invention, nitrate esters may also be employed prophylactically, to prevent or reduce anxiety, to aid sleep, and/or to enhance cognition.

25 Therapeutic compounds of the invention comprise at least one nitrate group. The nitrate group(s) can optionally be covalently bound to a carrier (e.g., an aromatic group, an aliphatic group, peptide, steroid, nucleobase, nucleoside, peptidomimetic, steroidomimetic, or nucleoside analogue, or the like). In addition to functioning as a carrier for the nitrate functionality, the carrier molecule can enable the compound to

30 traverse biological membranes and/or to be biodistributed preferentially, without excessive or premature metabolism. Further, in addition to functioning as a carrier for the nitrate

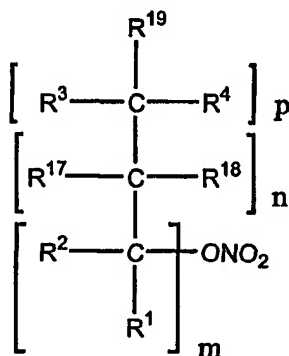
functionality, the carrier molecule can enable the compound to exert amplified analgesic, sedative, or anti-inflammatory effects through synergism with the nitrate functionality.

In one embodiment, the invention provides a method comprising administering to a subject an effective amount of a therapeutic compound which has at least one nitrate group and is capable of effecting analgesia. In another embodiment, the therapeutic compound is capable of mitigating inflammation. In a further embodiment, the therapeutic compound is capable of effecting sedation. In the respective embodiments, the therapeutic compound has the formula (Formula I):



wherein: E, F₁, F₂, G₁, G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions.

In further aspects of the invention, therapeutic compounds of the invention effect analgesia, effect sedation and/or mitigate inflammation in a subject to which the therapeutic compound is administered, and have the formula (Formula II).



in which: m, n, p are integers from 0 to 10; R^{3,17} are each independently hydrogen; a nitrate group; or A; R^{1,4} are each independently hydrogen; or A; where A is selected from: a substituted or unsubstituted aliphatic group (preferably a branched, or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain, which optionally may

contain O, S, NR⁶ and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S, NR⁶ and unsaturations in the ring, optionally bearing from 1 to 4

5 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between R¹ and R³ and/or between R¹⁷ and R⁴, which optionally may contain O, S, NR⁶ and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups); a substituted or unsubstituted aliphatic group (preferably a branched,

10 cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), containing carbonyl linkages (e.g., C=O, C=S, C=NOH), which optionally may contain O, S, NR⁶ and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a substituted or unsubstituted heterocyclic group; amino (including alkylamino, dialkylamino (including

15 cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy; R², R⁵, R¹⁸, R¹⁹ are optionally hydrogen; or A; or X-Y; where X is F, Br, Cl, NO₂, CH₂, CF₂, O, NH, NMe, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸,

20 S(O)₂OR⁹, PO₂HM, PO₃HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O), C(O)R¹², C(O)(OR¹³), PO₂H, PO₂M, P(O)(OR¹⁴), P(O)(R¹³), SO, SO₂, C(O)(SR¹³), SR⁵, SSR⁷ or SSR⁵; Y is F, Br, Cl, CH₃, CF₂H, CF₃, OH, NH₂, NHR⁶, NR⁶R⁷, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M,

25 SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₂HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O)R¹², C(O)(OR¹³), C(O)(SR¹³), SR⁵, SSR⁷ or SSR⁵, or does not exist; R⁶, R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶ are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO₂ substituents; or C₁ - C₆ connections to R¹

30 - R⁴ in cyclic derivatives; or are each independently hydrogen; a nitrate group; or A; M is

H, Na⁺, K⁺, NH₄⁺, N⁺H_kR¹¹_(4-k) where k is 0-3, or other pharmaceutically acceptable counterion.

Preferred therapeutic compounds for use in the invention include compounds in which R¹⁹ is X-Y. In a particularly preferred embodiment: R¹⁹ is X-Y and R⁵, R⁶, R⁸, R⁹, R¹⁰, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶ are the same or different alkyl groups containing 1-24 carbon atoms which may contain 1-4 ONO₂ substituents, or C₁ or C₂ connections to R¹ - R³ in cyclic derivatives; R¹ and R³ are the same or different and selected from H, C₁-C₄, alkyl chains, which may include one O, linking R¹ and R³ to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents; R² and R⁴, are the same or different and selected from H, a nitrate group, C₁-C₄ alkyl optionally bearing 1-3 nitrate group, and acyl groups (-C(O)R⁵); R⁷, R¹¹ are the same or different C₁-C₈, alkyl or acyl.

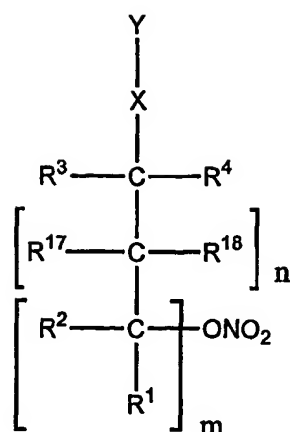
In certain embodiments in which R₁₉ is X-Y, m, p = 1, and n = 0. In other embodiments in which R₁₉ is X-Y, X is selected from CH₂, O, NH, NMe, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₃HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O), C(O)R¹², C(O)(OR¹³), PO₂M, P(O)(OR¹⁴), P(O)(R¹³), SO, SO₂, C(O)(SR¹³), SSR⁴. In another embodiment in which R₁₉ is X-Y, Y is selected from CN, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, SCN, SCN₂H₂(R¹⁵)₂, SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SR⁴, SO₂M, PO₃HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O)R¹², C(O)(SR¹³), SR⁵, SSR⁵, or does not exist.

In a further embodiment, X and/or Y contain a sulfur-containing functional group. In certain preferred embodiments, the compound of the invention comprises a heterocyclic functionality, more preferably, a nucleoside or nucleobase. In further preferred embodiments, the compound of the invention comprises a carbocyclic functionality, more preferably, a steroidal or carbohydrate moiety. In further preferred embodiments, the compound of the invention comprises a peptide or polypeptide moiety which may be a protein.

In another aspect of the invention, a therapeutic compound of the invention is represented by the formula (Formula III):

24

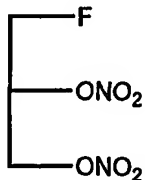
5



10 in which: m, n are 1-10; R^{1-18} , X , and Y have the meaning as defined above. In certain preferred embodiments, $R^6 - R^{16}$ are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO_2 substituents, or $C_1 - C_6$ connections to $R^1 - R^4$ in cyclic derivatives. In certain preferred embodiments, R^{18} is A and $m = n = 1$. In further preferred embodiments, therapeutic compounds of the

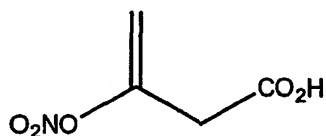
15 invention have a formula selected from (Formulae IIIa – IIIam):

IIIa



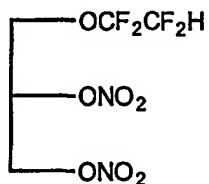
20

IIIb



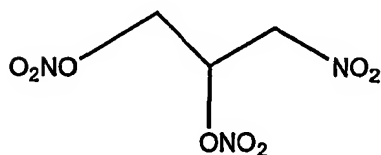
25

IIIc



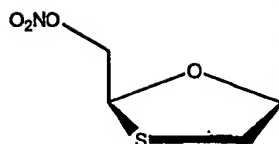
30

IIId

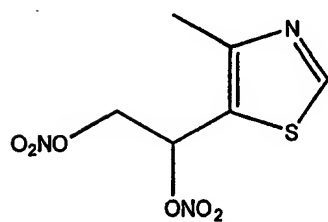


Nc1ccncc1

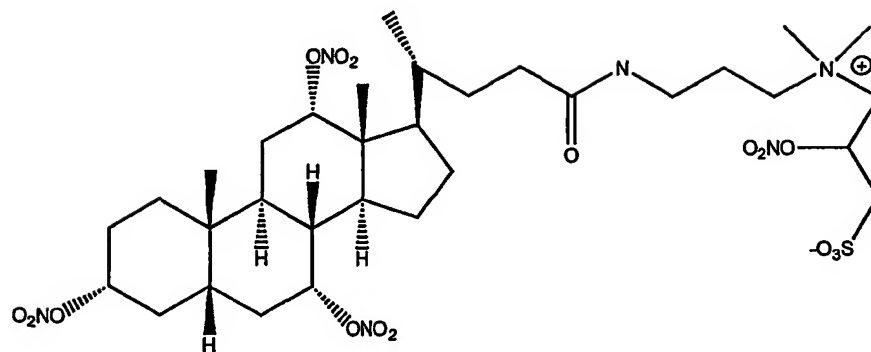
IIIe



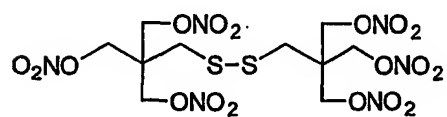
III f



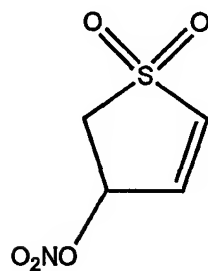
IIIg



IIIh

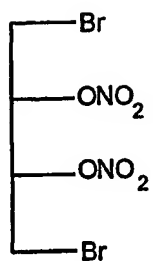


IIIi

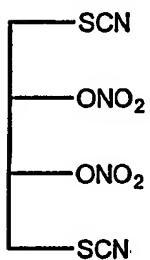


SUBSTITUTE SHEET (RULE 26)

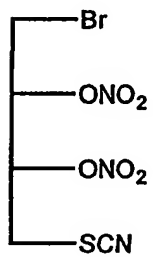
IIIj



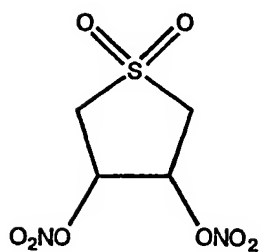
IIIk



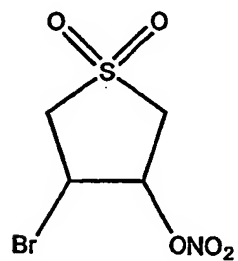
IIIl



IIIIm

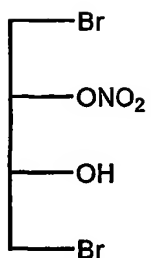


IIIIn



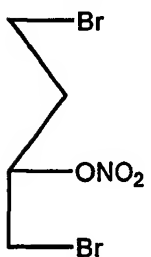
5

IIIo



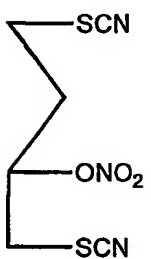
10

IIIp



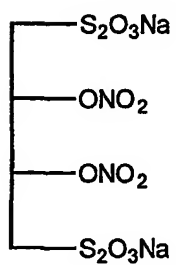
15

IIIq

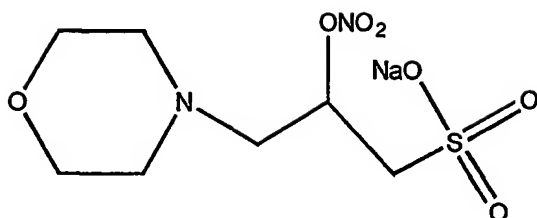


20

IIIr



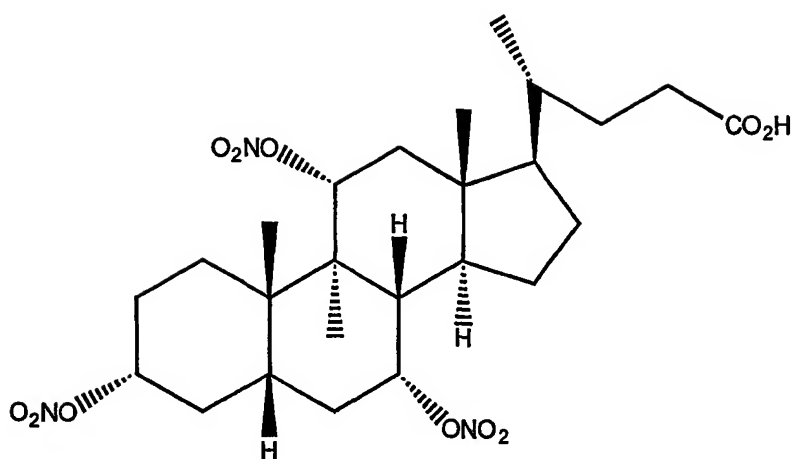
25

III_s

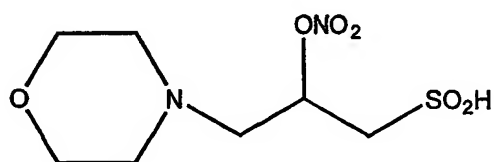
30

28

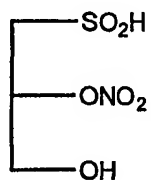
5

III_t

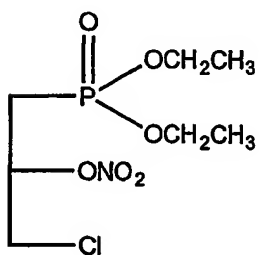
10

III_u

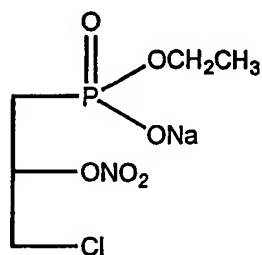
15

III_v

20

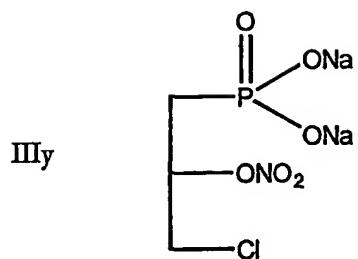
III_w

25

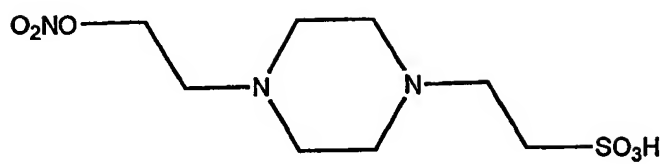
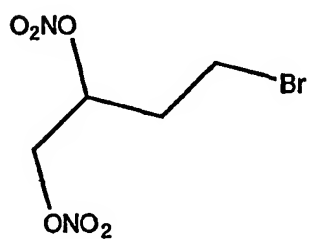
III_x

30

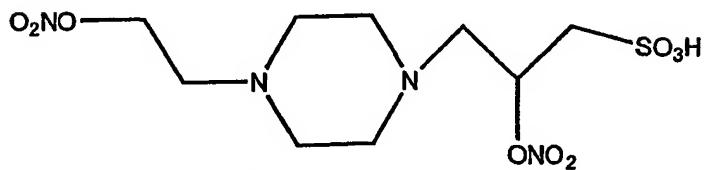
5



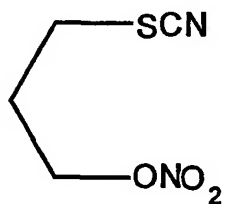
10

III_zIII_{aa}

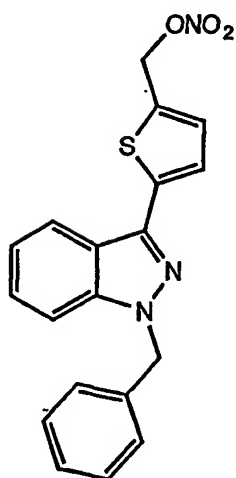
15

III_{ab}

20

III_{ac}

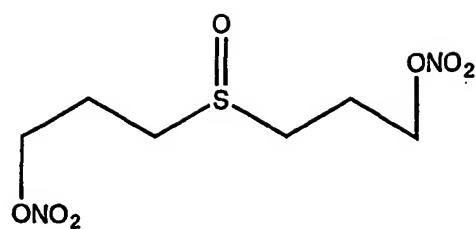
25

III_{ad}

30

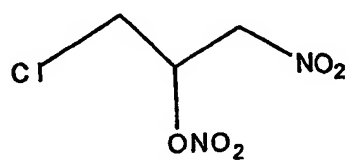
30

IIIae



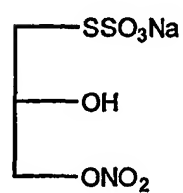
5

IIIaf



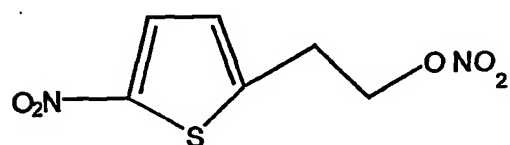
10

IIIag



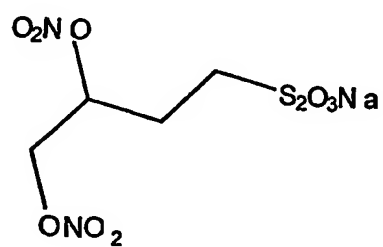
15

IIIah



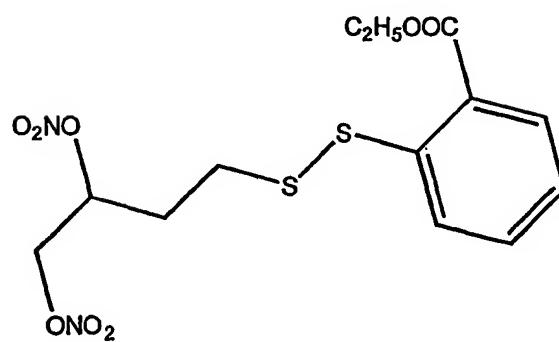
20

IIIai



25

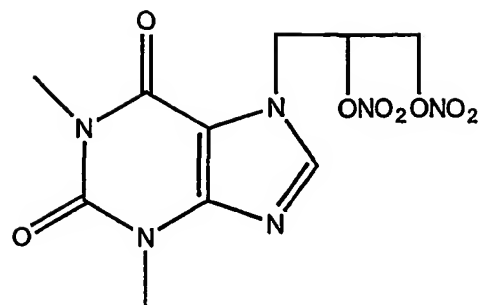
IIIaj



30

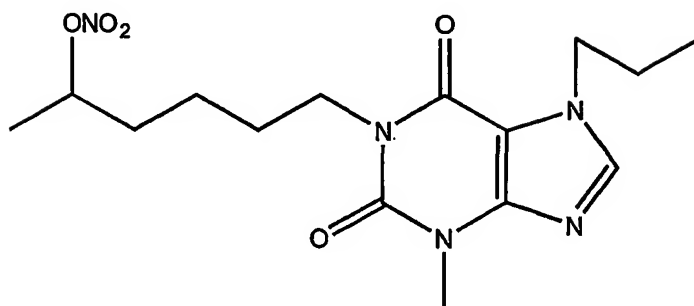
31

IIIak



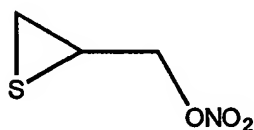
5

IIIal



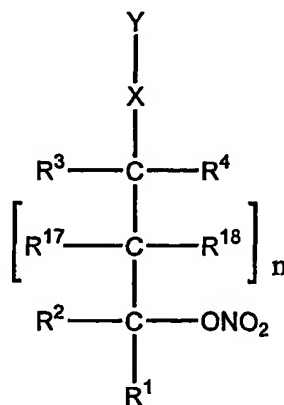
10

IIIam



15

In a further aspect of the invention, a therapeutic compound of the invention is represented by the formula (Formula IV):



20

25

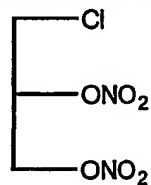
in which $n = 0$, X is CH_2 or does not exist, and Y is selected from, F, Br, Cl, CH_3 , CF_2H , CF_3 , OH, NH_2 , NHR^6 , NR^6R^7 , CN, NHOH , N_2H_3 , $\text{N}_2\text{H}_2\text{R}^{13}$, $\text{N}_2\text{HR}^{13}\text{R}^{14}$, N_3 , S, SCN, $\text{SCN}_2\text{H}_2(\text{R}^{15})_2$, $\text{SCN}_2\text{H}_3(\text{R}^{15})$, $\text{SC}(\text{O})\text{N}(\text{R}^{15})_2$, $\text{SC}(\text{O})\text{NHR}^{15}$, SO_3M , SH, SR^7 , SO_2M , $\text{S}(\text{O})\text{R}^8$, $\text{S}(\text{O})_2\text{R}^9$, $\text{S}(\text{O})\text{OR}^8$, $\text{S}(\text{O})_2\text{OR}^9$, PO_2HM , PO_3M_2 , $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$,

30

$P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)R^{12}$, $C(O)(OR^{13})$, $C(O)(SR^{13})$, SR^6 , SSR^7 or SSR^5 . R^2 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , and R^{16} are as defined above. In certain preferred embodiments, R^2 and R^4 are optionally H, a nitrate group or a connection to R^5 - R^{16} in cyclic derivatives.

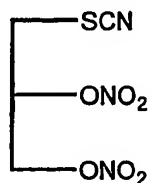
5 In certain preferred embodiments, a compounds of the invention is represented by the formula (Formulae IVa-IVt):

IVa

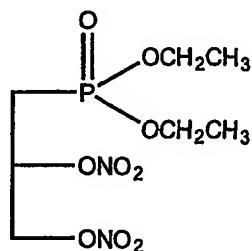


10

IVb

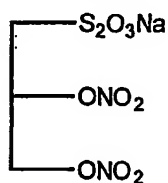


15 IVc

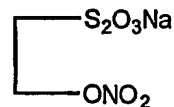


20

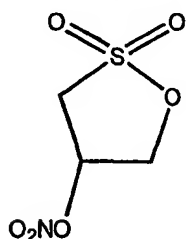
IVd



25 IVe



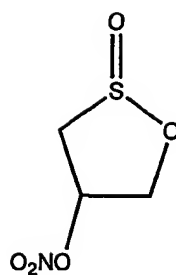
IVf



30

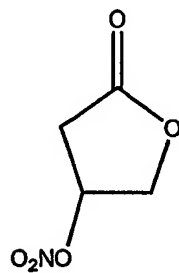
IVg

5



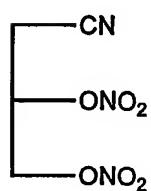
IVh

10



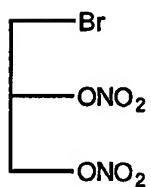
IVi

15



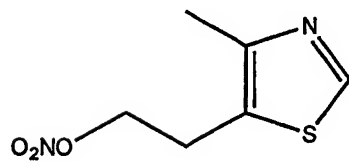
IVj

20



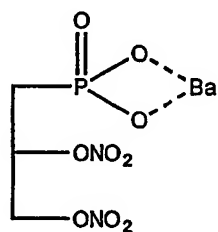
IVk

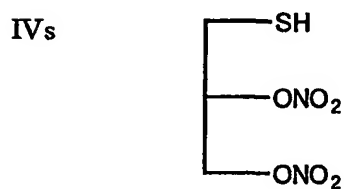
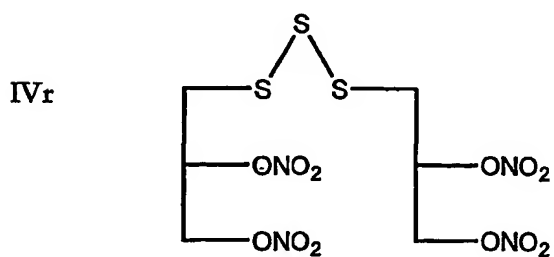
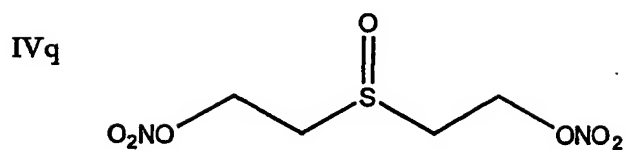
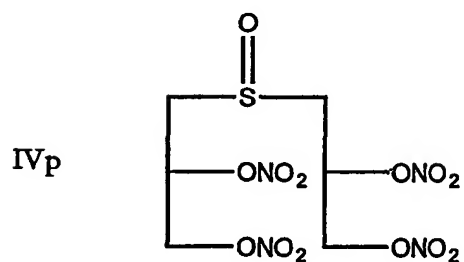
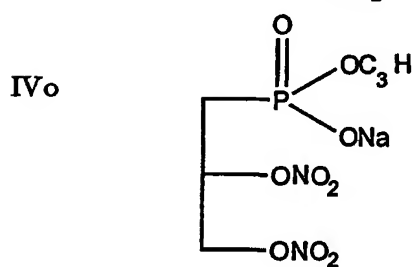
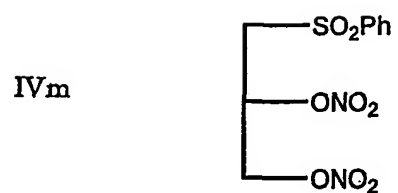
25



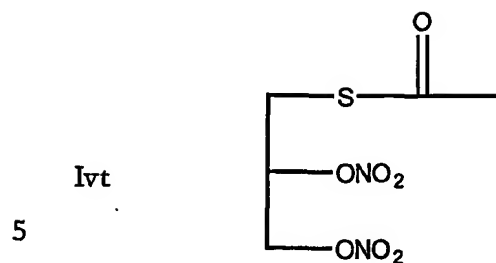
IVl

30

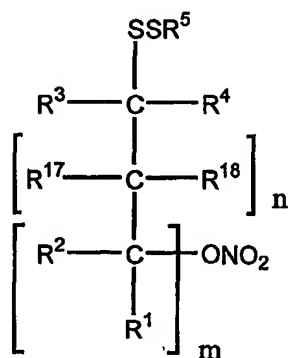




35

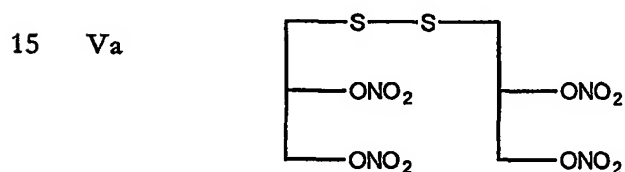


In a further aspect of the invention, compounds according to the invention are represented by the formula (Formula V):



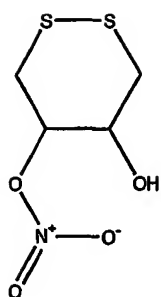
10 in which R^2 is optionally H or a connection to R^5 in cyclic derivatives, R^4 is H or a nitrate group, and R^5 is as described above.

In certain preferred embodiments, compounds of the invention are represented by the formula (Formulae Va-Vba):



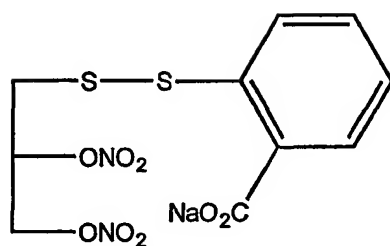
36

Vc



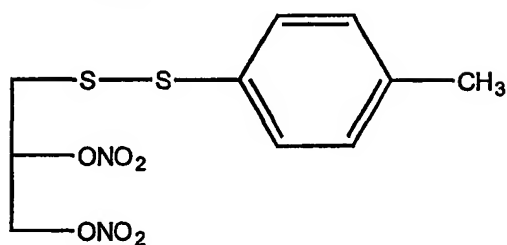
5

Vd



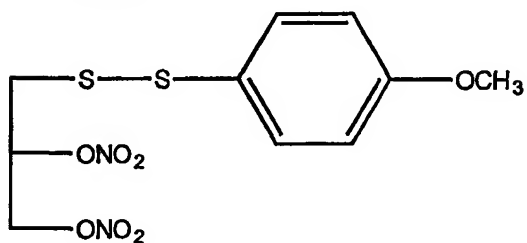
10

Ve



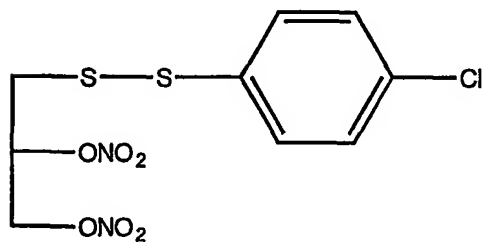
15

Vf



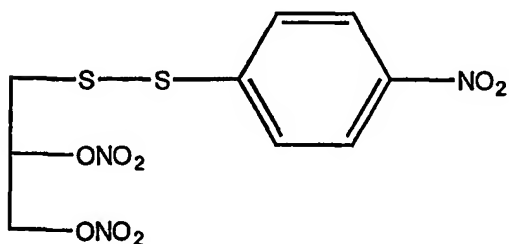
20

Vg



25

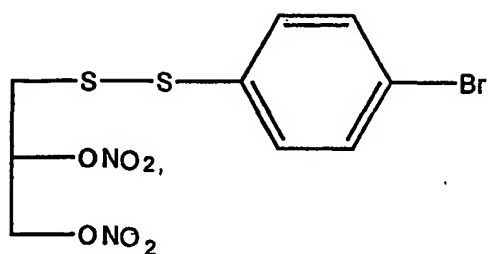
30 Vh



37

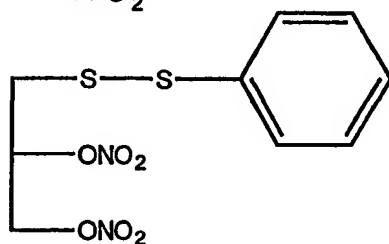
Vi

5



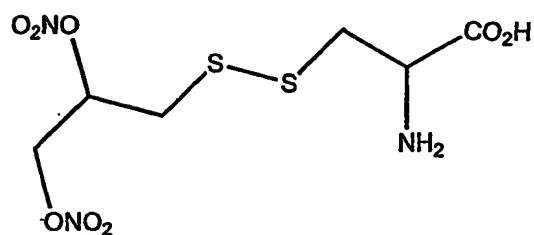
Vj

10

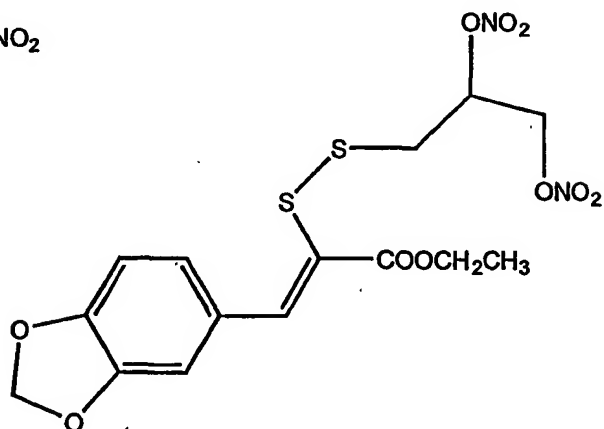


Vk

15

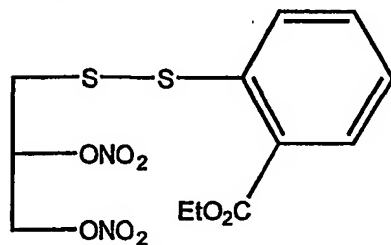


20 VI



25

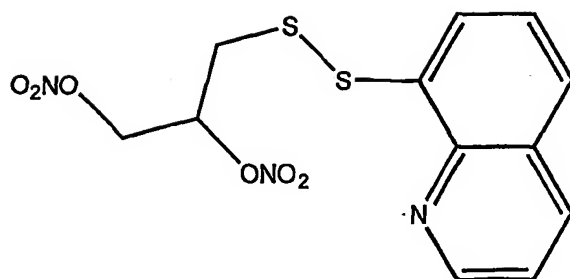
Vm



30

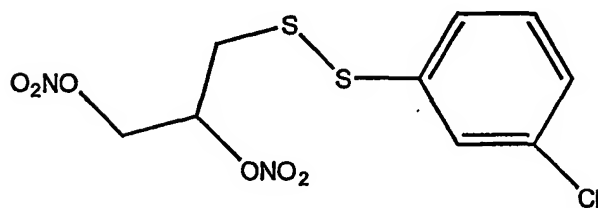
38

Vn



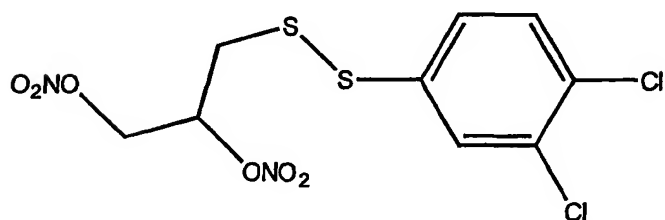
5

Vo



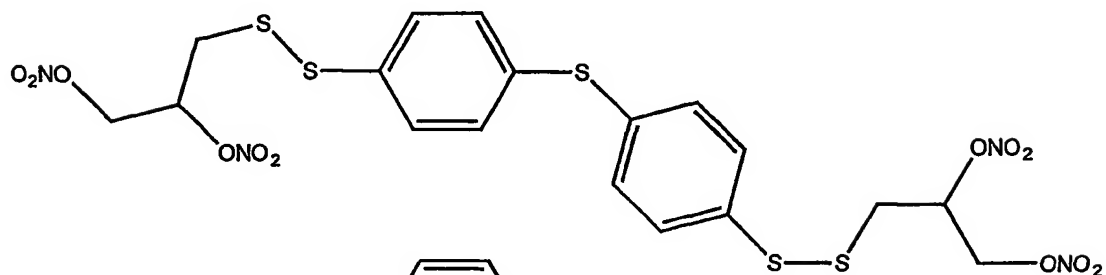
10

Vp



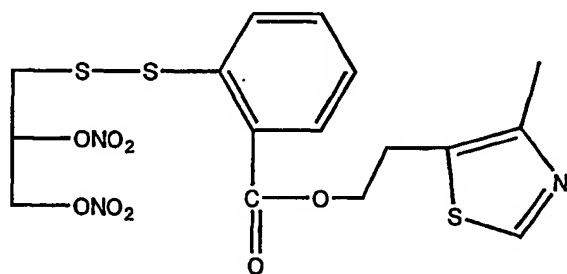
15

Vq



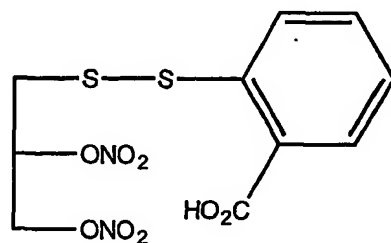
20

Vr



25

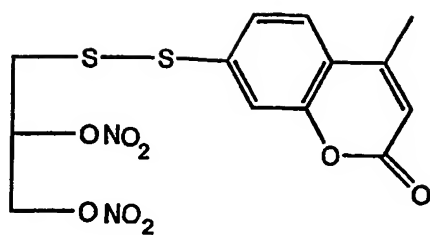
Vs



30

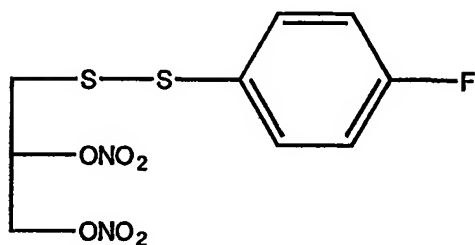
39

Vt



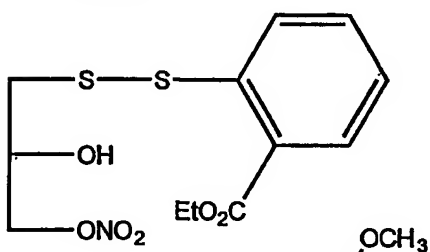
5

Vu



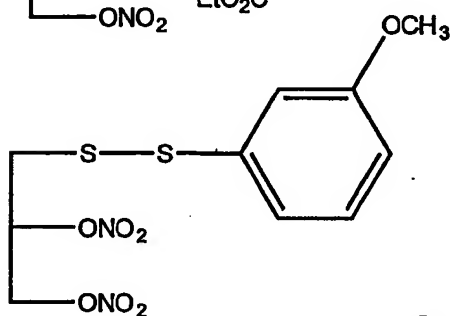
10

Vv



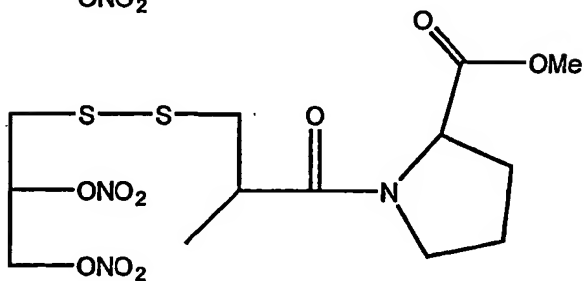
15

Vw



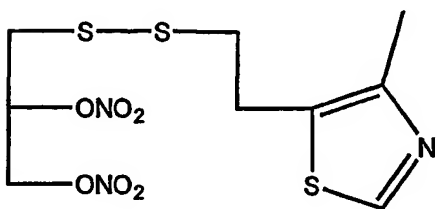
20

Vx



25

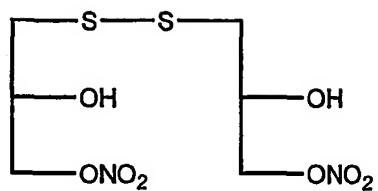
Vy



30

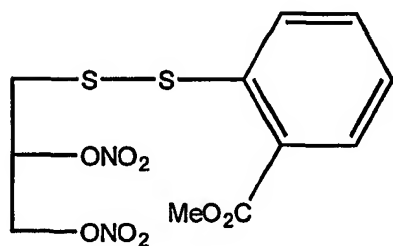
40

Vz



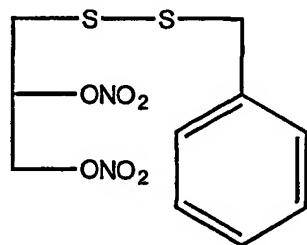
5

Vaa



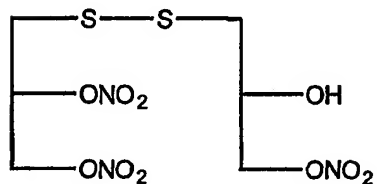
10

Vab



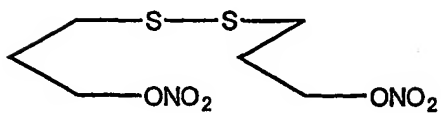
15

Vac



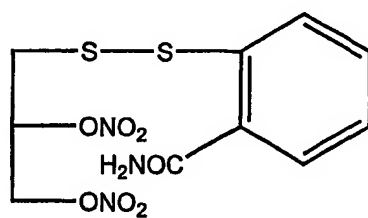
20

Vad



25

Vae

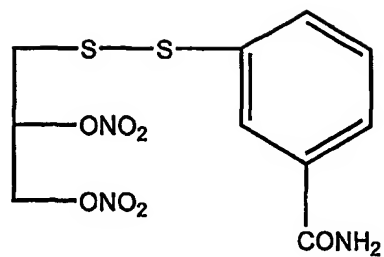


30

41

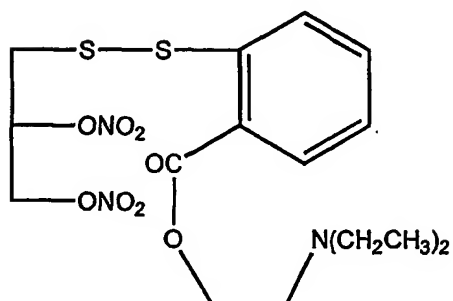
Vaf

5



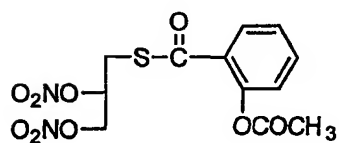
Vag

10

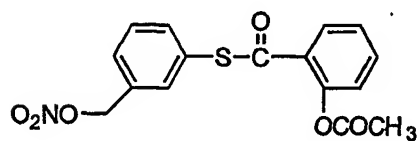


15

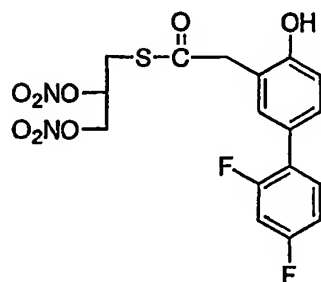
Vah



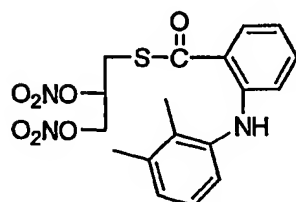
20 Vai



25 Vaj

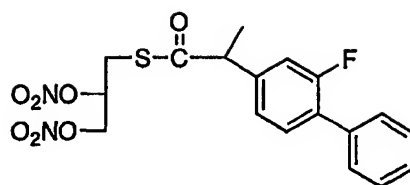


30 Vak



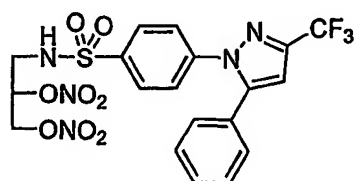
42

Val



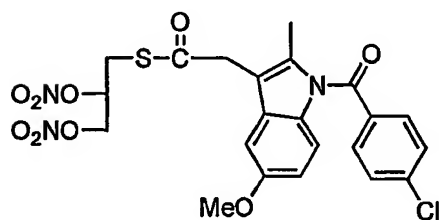
5

Vam



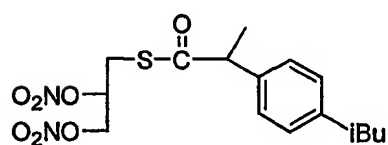
10

Van



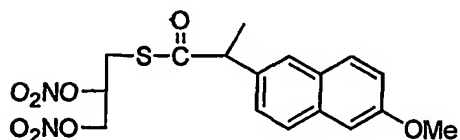
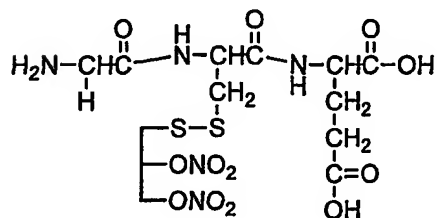
15

Vao



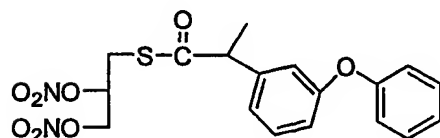
20

Vap

25 V_{aq}

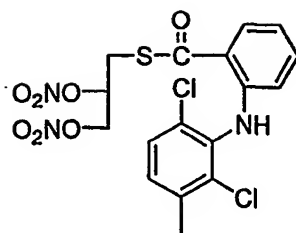
30

Var



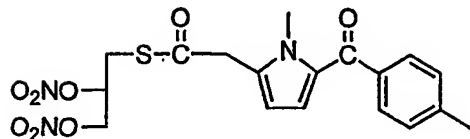
Vas

5



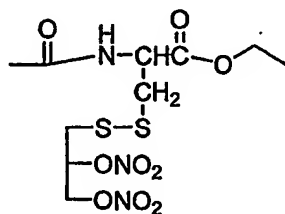
Vat

10



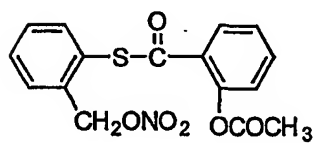
Vau

15



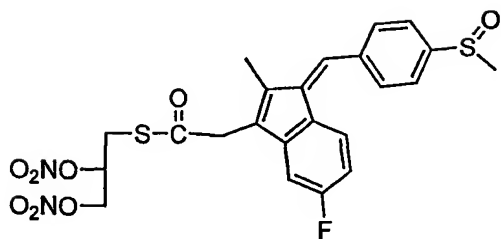
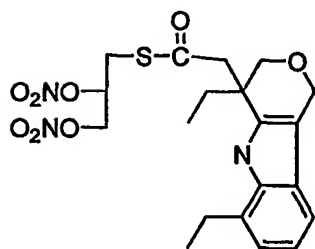
Vav

20

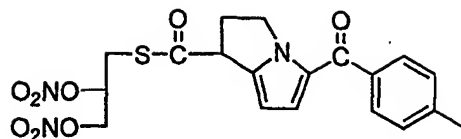


Vaw

25 Vax

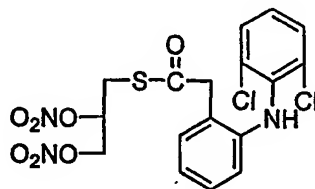


Vay

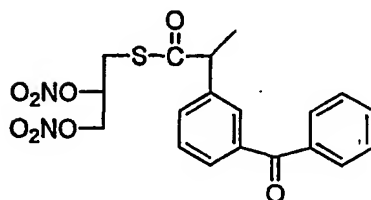


5

Vaz



10 Vba



In another aspect, the invention provides novel compounds which can be represented by structures of Formula III, Formula IV, and Formula V. Table 1 lists data associated with these compounds using art-recognized characterization techniques.

Further, the invention provides novel pharmaceutical compositions comprising a therapeutic compound (nitrate ester) of the invention and a pharmaceutically acceptable vehicle.

Table 1	¹ H NMR	¹³ C NMR
IIIa	(CDCl ₃): 5.34-5.57 (1H, dm, ³ J _{HF} 20.6), 4.53-4.87 (4H, superposition several multiplets, O ₂ NO-CH ₂ + CH ₂ F, ² J _{HF} 46.7, ⁴ J _{HF} 0.66)	(CDCl ₃): 79.47 (d, ¹ J _{CF} 177), 76.73 (d, ² J _{CF} 20.6), 67.84 (d, ³ J _{CF} 6.87)
IIIc	(CDCl ₃): δ 5.7 (1H, t, ² J _{HF} 54), 5.45 (1H, m), 4.5-4.9 (2H, m), 4.15-4.35 (1H, m)	(CDCl ₃): δ 75.55, 68.05, 60.76
IIId	(CDCl ₃): δ 5.46 (1H, m), 4.80-4.87 (1H, dd, J 3.5, 12.9), 4.65-4.72 (1H, dd, J 6.2, 12.9), 3.7-3.8 (2H, m)	(CDCl ₃): δ 77.24, 68.57, 39.86
IIIf	(CDCl ₃) δ 8.72 (s, 1H), 5.38 (t, 1H), 4.6 (d, 2H),	-

	2.45 (s, 3H)	
IIIg	(DMSO-d ₆) CHONO ₂ only: δ 4.8-5.8	(DMSO-d ₆) CONO ₂ only: δ 85.68, 84.17, 82.47, 76.50
IIIh	(CD ₃ OD) δ 4.85 (3H, m), 3.5 (1H, m)	(CD ₃ OD) δ 70.61, 36.74
IIIi	(CDCl ₃): δ 6.95 (dd, 1H), 6.71 (dd, 1H), 6.09 (m, 1H), 3.80 (dd, 1H), 3.32 (dd, 1H)	(CDCl ₃): δ 137.9, 132.5, 76.6, 52.9
IIIj	(CDCl ₃): δ 5.62 (2H, m), 3.60 (4H, m)	(CDCl ₃): δ 77.87, 25.22
IIIk	(CD ₃ CN): δ 3.45 (m, 2H), 5.72 (m, 2H)	(CD ₃ CN): δ 79.98, 28.87
IIIl	-	(CD ₃ CN): δ 79.48, 33.45, 28.47
IIIm	(DMSO-d ₆): δ 5.97 (m, 2H), 3.80 (m, 4H)	(DMSO-d ₆): δ 78.84, 52.60
III n	(CDCl ₃): δ 5.73 (m, 1H), 4.62 (m, 1H), 3.96-3.77 (m, 1H), 3.58-3.32 (m, 1H)	(CDCl ₃): δ 81.47, 57.85, 53.50, 38.75
IIIo	-	(CDCl ₃): δ 81.24, 69.79, 33.26, 27.24
IIIp	(CDCl ₃): δ 5.36 (m, 1H), 3.11-3.60 (m, 4H), 2.33 (m, 2H)	(CDCl ₃): δ 78.92, 33.66, 30.64, 27.36
IIIq	(CDCl ₃): δ 5.47 (m, 1H), 3.53-3.05 (m, 4H), 2.29 (m, 2H)	(CDCl ₃): δ 81.32, 37.12, 32.97, 30.98
IIIw ³¹ P(CDCl ₃ , 162 MHz) 24.60	(CDCl ₃ , 300 MHz): 5.31-5.45 (m, 1H), 3.92-4.08 (m, 4H), 3.63-3.81 (m, 2H), 2.03-2.30 (m, 2H), 1.16-1.24 (superposition of 2 t, 6H, J 7)	(CDCl ₃ , 75 MHz): 76.83, 62.15 (d, J 6.37), 43.77 (d, J 8.95), 27.08 (d, J 142.00), 15.99 (d, J 5.88)
IIIx ³¹ P (CD ₃ OD, 122 MHz) 17.62	(CD ₃ OD, 300 MHz): 5.38-5.63 (m, 1H), 3.75-4.25 (superposition of 2 m, 4H), 1.88-2.20 (m, 2H), 1.12-1.28 (t, 3H)	(CD ₃ OD, 75 MHz): 81.14, 61.17 (d, J 5.41), 45.56 (d, J 5.94), 29.35 (d, J 131.74), 17.00 (d, J 6.75)
IIIak	¹ H (CD ₃ CN, 300 MHz): 7.8 (s, 1H), 5.75-5.85 (m, 1H), 4.90-4.97 (dd, 1H, J 12.87, 3.39), 4.54-4.76 (m, 3H), 3.46 (s, 3H), 3.27 (s, 3H)	¹³ C (CD ₃ CN, 75 MHz) (for polynitrated chain): 78.51, 70.58, 46.01, 30.10, 28.18
Ivi	(CDCl ₃): δ 5.45 (1H, m), 4.83 (1H, dd), 4.65 (1H,	(CD ₃ OD): δ 116.44, 75.37,

	dd), 2.9 (2H, m)	71.20, 19.19
Ivk	(CDCl ₃) δ 8.55 (s, 1H), 4.55 (t, 2H), 3.15 (t, 2H), 2.37 (s, 3H)	(CDCl ₃) δ 150.9, 150.7, 125.3, 72.53, 24.47, 15.18
Ivm	(CDCl ₃): δ 7.5-8.0 (arom, 5H), 5.7 (1H, m), 4.94 (1H, dd), 4.62 (1H, dd), 3.5 (2H, m)	(CDCl ₃): δ 135.45, 134.79, 129.81, 27.95, 73.08, 70.04, 54.73
Ivs	¹ H-NMR(CDCl ₃ , 300 MHz): 5.23-5.32 (1H, m), 4.87 (1H, dd, J 12.82, 3.22), 4.68 (1H, dd, J 12.83, 6.09), 2.77-2.94 (2H, m), 1.66 (1H, t, J 9.07)	¹³ C-NMR: (CDCl ₃ , 75.48 MHz): 79.39, 69.30, 23.68
Ivt	¹ H-NMR(CDCl ₃ , 300 MHz): 5.29-5.38 (1H, m), 4.76 (1H, dd, J 12.94, 3.11), 4.55 (1H, dd, J 12.94, 6.37), 3.30 (1H, dd, J 14.06, 5.98), 3.13 (1H, dd, J 14.61, 6.35)	¹³ C-NMR: (CDCl ₃ , 75.48 MHz): 194.10, 77.00, 69.79, 30.42, 27.78
Vb	(CDCl ₃) δ 5.56 (m, 2H), 3.38-2.95 (m, 4H)	(CD ₃ OD) δ 85.93, 32.77
Vc	(CDCl ₃): δ 5.85-5.91 (1H, m), 4.50-4.58 (1H, m), 3.22-3.29 (1H, dd, J 5.47, 12.78), 2.97-3.05 (1H, dd, J 4.6, 11.88), 2.82-2.90 (1H, dd, J 2.87, 12.78), 2.74-2.83 (1H, dd, J 3.15, 11.9)	(CDCl ₃): δ 87.6, 74.96, 36.20, 31.54
Ve	(CDCl ₃): δ 7.44-7.51 (m, arom 2H), 7.17-7.24 (d, arom 2H, J 7.91), 5.47-5.59 (m, 1H), 4.83-4.93 (dd, 1H, J 12.81, 2.78), 4.57-4.67 (dd, 1H, J 12.82, 5.71), 3.02-3.12 (dd, 1H, J 14.48, 6.01), 2.9-2.99 (dd, 1H, J 14.47, 7.72), 2.38 (s, 3H)	(CDCl ₃): δ 21.53, 36.78, 69.82, 77.68, 130.52, 130.62, 132.55, 139.23
Vf	(CDCl ₃): δ 7.48-7.57 (m, arom 2H), 7.48-7.57 (m, arom 2H), 5.49-5.59 (m, 1H), 4.84-4.93 (dd, 1H, J 12.79, 2.79), 4.58-4.68 (dd, 1H, J 12.79, 5.75), 3.84 (s, 3H), 3.02-3.12 (dd, 1H, J 14.47, 5.8), 2.89-2.99 (dd, 1H, J 14.46, 7.99).	(CDCl ₃): δ 36.57, 55.87, 69.75, 77.76, 115.47, 126.71, 133.76, 160.94.
Vg	(CDCl ₃): δ 7.47-7.54 (m, arom 2H), 7.32-7.38 (m,	(CDCl ₃): δ 36.87, 69.80, 77.5,

	arom 2H), 5.45-5.55 (m, 1H), 4.84-4.97 (dd, 1H, J 12.86, 2.92), 4.58-4.68 (dd, 1H, J 12.86, 5.68), 2.91-3.11 (m, 2H).	129.98, 130.85, 134.51, 134.79.
Vh	(CDCl ₃): δ 8.21-8.27 (m, arom 2H), 7.67-7.74 (m, arom 2H), 5.44-5.54 (m, 1H), 4.86-4.94 (dd, 1H, J 12.92, 3.11), 4.61-4.70 (dd, 1H, J 12.92, 5.56), 3.01-3.16 (m, 2H).	(CDCl ₃): δ 37.24, 69.75, 77.41, 124.82, 127.26, 144.83
Vi	(CDCl ₃): δ 7.40-7.55 (m, arom 4H), 5.44-5.55 (m, 1H), 4.85-4.92 (dd, 1H, J 12.87, 2.91), 4.60-4.70 (dd, 1H, J 12.86, 5.66), 2.92-3.11 (m, 2H).	(CDCl ₃): δ 36.87, 69.79, 77.48, 122.72, 130.93, 132.90, 135.15.
Vj	(CDCl ₃): δ 7.55-7.62 (d, arom 2H, J 7.16), 7.29-7.44 (m, arom 3H), 5.46-5.58 (m, 1H), 4.82-4.92 (dd, 1H, J 12.85, 2.79), 4.57-4.67 (dd, 1H, J 12.86, 5.67), 3.01-3.13 (dd, 1H, J 14.51, 6.24), 2.92-3.02 (dd, 1H, J 14.52, 7.4)	(CDCl ₃): δ 36.97, 69.88, 77.61, 128.60, 129.50, 129.85, 136.02
Vk	(CDCl ₃ , 300 MHz): 8.09 (1H, dd, J 8.12, 0.36), 8.02 (1H, dd, J 7.8, 1.15), 7.51-7.59 (1H, m, J 7.24, 1.44), 7.21-7.29 (1H, m, J 7.35, 0.54), 5.40-5.49 (1H, m), 4.70-4.78 (1H, dd, J 13.04, 2.95), 4.33-4.45 (3H, m, superposition of 1H from CH ₂ -ONO ₂ and quartet from O-CH ₂ -CH ₃), 2.66-2.87 (2H, quartet, J 6.92), 1.39 (3H, t, J 7.14)	(CDCl ₃ , 75.48 MHz): 166.21, 140.41, 132.78, 131.46, 127.63, 125.48, 77.25, 71.03, 61.42, 32.51, 28.29, 14.17
VI	(CDCl ₃ , 300MHz): δ 7.97 (s, 1H), 7.48-7.51 (d, 1H), 7.17-7.22 (m, 1H), 6.84-6.89 (d, 1H), 6.05 (s, 2H), 5.48-5.58(m, 1H), 4.81-4.89 (dd, 1H), 4.53-4.61 (dd, 1H), 4.25-4.4 (m, 2H), 3.05-3.10 (m, 2H), 1.35-1.45 (t, 3H).	(CDCl ₃ , 75.48 MHz): δ 14.17, 36.27, 62.15, 69.71, 76.57, 101.77, 108.27, 110.31, 125.20, 127.55, 127.87, 146.87, 147.811, 149.76, 165.89

V _m	(CDCl ₃ , 300MHz): δ 8.04-8.11 (m, arom 2H), 7.55-7.62 (m, arom 1H), 7.30-7.34 (m, arom 1H), 5.43-5.54 (m, 1H), 4.88-4.97 (dd, 1H, J 12.95, 2.79), 4.62-4.71 (dd, 1H, J 12.94, 5.35), 4.45-4.39 (q, 2H, J 7.12), 2.92-3.08 (m, 2H), 1.39-1.47 (t, 3H, J 7.13)	(CDCl ₃ , 75.48 MHz): δ 14.17, 26.23, 35.95, 61.55, 69.54, 77.24, 125.56, 125.92, 127.91, 131.52, 132.93, 139.56, 166.189
V _n	(CDCl ₃ , 300MHz): δ 8.92-8.97 (m, arom 1H), 8.07-8.23 (m, arom 2H), 7.46-7.77 (m, arom 3H), 5.53-5.62 (m, 1H), 4.91-4.99 (dd, 1H, J 12.98, 2.77), 4.61-4.7 (dd, 1H, J 12.98, 5.35), 3-3.18 (m, 2H)	(CDCl ₃ , 75.48 MHz): δ 36.06, 69.62, 70.24, 77.42, 121.97, 125.65, 126.43, 126.70, 128.52, 135.31, 136.37, 145.62, 149.65
V _o	(CDCl ₃ , 300MHz): δ 7.55-7.59 (m, arom 1H), 7.40-7.45 (m, arom 1H), 7.25-7.36 (m, arom 2H), 5.45-5.55 (m, 1H), 4.85-4.95 (dd, 1H, J 12.98, 3), 4.59-4.69 (dd, 1H, J 12.89, 6.67), 2.95-3.12 (m, 2H)	(CDCl ₃ , 75.48 MHz): δ 37.10, 69.87, 77.44, 126.80, 128.38, 128.52, 130.83, 135.76, 138.01
V _p	(CDCl ₃ , 300MHz): δ 7.65-7.67 (d, arom 1H J 2.15), 7.44-7.47 (d, arom 1H, J 7.42), 7.28-7.49 (m, arom 1H), 5.46-5.54 (m, 1H), 4.87-4.94 (dd, 1H, J 12.89, 2.99), 4.61-4.68 (dd, 1H, J 12.88, 5.65), 2.95-3.12 (m, 2H)	(CDCl ₃ 75.48 MHz): δ 36.59, 69.32, 76.90, 127.49, 129.86, 131.04, 132.31, 133.59, 135.65
V _q	(CDCl ₃ , 300MHz): δ 7.2-7.6 (m, arom 8H), 5.42-5.56 (m, 2H), 4.82-4.95 (dd, 2H), 4.55-4.67 (dd, 2H), 2.93-3.15 (m, 4H)	
V _r	(CDCl ₃ , 300MHz): δ 8.63 (s, arom 1H), 8.01-8.13 (m, arom 2H), 7.57-7.65 (m arom 1H), 7.26-7.37 (m, arom 1H), 5.42-5.51 (m, arom 1H), 4.88-4.97 (dd, 1H, J 12.9, 2.7), 4.62-4.71 (dd, 2H, J 12.9, 5.1), 4.49-4.57 (t, 2H, J 6.6), 3.23-3.32 (t, 2H, J 6.6), 2.91-3.08 (m, 2H), 2.45 (s, 3H)	
V _s	(d ₆ -acetone), 300 MHz): 8.16-8.23 (1H, d, J 7.72), 8.07-8.13 (1H, dd, J 7.72, 1.11), 7.63-7.72 (1H, m, J 8.38, 1.26), 7.33-7.42 (1H, m, J 7.33), 5.67-5.76 (1H, m), 5.09-5.17 (1H, dd, J 12.91, 2.68), 4.83-4.93 (1H, dd, J 12.91, 5.91), 3.18-3.32 (2H, m)	(d ₆ -acetone, 100.62 MHz): 167.74, 134.01, 132.64, 126.62, 126.21, 78.90, 71.69, 36.79
V _t	(CDCl ₃ , 300MHz): δ 7.53-7.59 (d, arom 1H, J	(CDCl ₃ , 75.48 MHz): δ 19.01,

	8.35), 7.46-7.49 (d, arom 1H, J 1.82), 7.37-7.43 (m, arom 1H), 6.24 (s, 1H), 5.46-5.55 (m, 1H), 4.86-4.94 (dd, 1H, 12.92, 2.92), 4.61-4.69 (dd, 1H, J 12.93, 5.75), 3.0-3.15 (m, 2H), 2.42 (s, 3H)	37.10, 70.01, 77.46, 115.27, 115.34, 119.52, 123.07, 125.83, 141.13, 152.38, 154.25, 160.51
Vu	(CDCl ₃ , 300MHz): δ 7.55-7.58 (m, arom 1H), 7.40-7.45 (m, arom 1H), 7.27-7.32 (m, arom 2H), 5.46-5.54 (m, 1H), 4.86-4.94 (dd, 1H, J 12.88, 2.92), 4.59-4.68 (dd, 1H, J 12.88, 5.65), 2.95-3.13 (m, 2H)	(CDCl ₃ , 75.48 MHz): δ 37.08, 69.83, 77.40, 126.80, 128.40, 128.54, 130.83, 135.78, 137.98
Vv	(CDCl ₃ , 300 MHz): 8.09-8.14 (1H, d, J 8.15), 7.98-8.04 (1H, dd, J 7.75, 0.91), 7.52-7.59 (1H, m, J 8.22, 1.19), 7.22-7.28 (1H, m, J 7.65), 4.53-4.61 (1H, dd, J 11.33, 3.63), 4.42-4.50 (1H, m, J 11.33, 6.27), 4.32-4.41 (2H, qu., J 7.13), 4.16-4.25 (1H, m), 2.74-2.84 (2H, m), 2.55-2.73 (1H, br.s.), 1.34-1.42 (3H, t, J 7.13)	(CDCl ₃ , 75.48 MHz): 166.34, 140.25, 132.90, 131.49, 127.67, 125.60, 74.34, 66.83, 61.53, 40.89, 14.20
Vx	(CDCl ₃ , 300MHz): δ 5.49-5.61 (m, 1H), 4.88-4.90 (m, 1H), 4.62-4.74 (m, 1H), 4.40-4.55 (m, 1H), 3.59-3.78 (m, superpos. 5H), 2.69-3.21 (m, superpos. 5H), 2.14-2.29 (m, 1H), 1.90-2.12 (m, 3H), 1.22-1.28 (d, 3H)	(CDCl ₃ , 75.48 MHz): δ 17.39, 25.20, 29.40, 36.41/36.51, 38.55, 41.94/42.19, 47.32, 52.56, 59.12, 69.90/70.29, 77.89/78.00, 173.01, 173.48
Vy	(CDCl ₃ , 300 MHz): δ 8.5 (s, 1H), 2.94-3.02 (t, 2H), 2.62-2.72 (q, 2H), 2.33 (s, 3H), 1.38-1.45 (t, 1H).	(CDCl ₃ , 75.48 MHz): δ 15.41, 26.41, 31.51, 39.72, 129.36, 149.90
Vz	(CD ₃ CN, 300 MHz): 4.60-4.68 (1H, dd, J 11.44, 3.36), 4.43-4.52 (1H, dd, J 11.35, 6.97), 4.11-4.21 (1H, m), 2.80-3.00 (2H, m)	(CD ₃ CN, 75.48 MHz): 76.33, 67.34, 42.74
Vaa	(CDCl ₃ , 300MHz): δ 8.05-8.15 (m, arom 2H), 7.55-7.65 (m, arom 1H), 7.25-7.35 (m, arom 1H), 5.40-5.55 (m, 1H), 4.85-4.95 (dd, 1H), 4.60-4.70 (dd, 1H), 3.95 (s, 3H), 2.90-3.10 (m, 2H)	(CDCl ₃ , 5.48 MHz): δ 36.42, 52.87, 69.98, 77.69, 126.07, 126.45, 128.01, 132.06, 133.53, 140.161, 167.10

Vab	(CDCl ₃ , 300MHz): δ 7.30-7.39 (m, arom 5H), 5.28-5.37 (m, 1H), 4.72-4.78 (dd, 1H, J 12.86, 2.79), 4.45-4.53 (dd, 1H, J 12.86, 5.9), 3.95 (s, 2H), 2.44-2.52 (dd, 1H, J 14.38, 6.1), 2.33-2.42 (dd, 1H, J 14.38, 7.34)	(CDCl ₃ , 75.48 MHz): δ 32.01, 35.997, 43.66, 69.89, 77.72, 128.33, 129.22, 129.81, 137.22
Vac	¹ H-NMR(CDCl ₃ , 300 MHz): 5.48-5.58 (1H, m), 4.89 (1H, ddd, J 12.91, 2.91, 1.21), 4.56-4.70 (2H, m), 4.48 (1H, dd, J 11.41, 6.4), 4.16-4.26 (1H, m), 2.94-3.12 (2H, m), 2.81-2.91 (2H, m)	¹³ C-NMR: (CDCl ₃ , 75.48 MHz): 77.30, 74.22, 69.46, 69.38, 66.90, 66.78, 42.12, 41.99, 36.82, 36.59
Vad	(CDCl ₃ , 400 MHz): 5.55-5.65 (m, 1H), 4.87-4.94 (dd, 1H, J 12.94, 2.94), 4.62-4.70 (m, 1H, J 12.88), 3.13-3.30 (m, 2H)	(CDCl ₃ , 400 MHz): 76.74, 69.46/69.42, 36.65/36.63
Vah	(CDCl ₃ , 300 MHz): 8.21 (1H, dd, J 8.01, 1.62), 7.65 (1H, td, J 7.9, 1.63), 7.39 (1H, td, J 6.7, 1.1), 7.14 (1H, dd, J 8.07, 1.1), 5.29-5.38 (1H, m), 4.76 (1H, dd, J 12.94, 3.11), 4.55 (1H, dd, J 12.94, 6.37), 3.30 (1H, dd, J 14.06, 5.98), 3.13 (1H, dd, J 14.61, 6.35), 2.33 (3H, s)	(CDCl ₃ , 75.48 MHz): 169.13, 164.58, 150.28, 136.05, 134.32, 126.44, 126.27, 124.19, 77.00, 69.79, 30.42, 20.79

It will be noted that the structure of some of the compounds of this invention include asymmetric carbon atoms. It is to be understood accordingly that the isomers (e.g., enantiomers, diastereomers) arising from such asymmetry are included within the scope of this invention. Such isomers can be obtained in substantially pure form by classical separation techniques and by asymmetric synthesis (for example, see below in Example 21). Unless expressly noted to the contrary, compounds referred to herein shall be construed to include both the R and S stereoisomers at each stereogenic centre.

In certain embodiments, a therapeutic compound of the invention comprises a cation (i.e., in certain embodiments, one of X or Y includes a cation, e.g., in the compound of formula IVd). If the cationic group is a proton, then the compound is considered an acid. If the proton is replaced by a metal ion or its equivalent, the compound is a salt.

Pharmaceutically acceptable salts of the therapeutic compound are within the scope of the invention. For example, M can be a pharmaceutically acceptable alkali metal (e.g., Li, Na, K), ammonium, alkaline earth metal (e.g., Ca, Ba, Mg), higher valency cation, or polycationic counter ion (e.g., polyammonium cation) (see e.g., Berge *et al.* (1977)). It will be appreciated that the stoichiometry of an anionic portion of the compound to a salt-forming cation will vary depending on the charge of the anionic portion of the compound and the charge of the counterion. Preferred pharmaceutically acceptable salts include a sodium, potassium, or calcium salt, but other salts are also contemplated within their pharmaceutically acceptable range.

Therapeutic compounds of the invention can be administered in a pharmaceutically acceptable vehicle. As used herein "pharmaceutically acceptable vehicle" includes any and all solvents, excipients, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like which are compatible with the activity of the compound and are physiologically acceptable to the subject. An example of a pharmaceutically acceptable vehicle is buffered normal saline (0.15 M NaCl). The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the therapeutic compound, use thereof in the compositions suitable for pharmaceutical administration is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Carrier or substituent moieties useful in the present invention may also include moieties which allow a therapeutic compound to be selectively delivered to a target organ. For example, delivery of a therapeutic compound to the brain may be enhanced by a carrier moiety using either active or passive transport (a "targeting moiety"). Illustratively, the carrier molecule may be a redox moiety, as described in, for example, U.S. Patents 4,540,654 and 5,389,623, both to Bodor. These patents disclose drugs linked to dihydropyridine moieties which can enter the brain, where they are oxidized to a charged pyridinium species which is trapped in the brain. Thus drugs accumulate in the brain. Other carrier moieties include compounds, such as amino acids or thyroxine, which can be passively or actively transported *in vivo*. Such a carrier moiety can be metabolically removed *in vivo*, or can remain intact as part of an active compound. Structural mimics

of amino acids (and other actively transported moieties) including peptidomimetics, are also useful in the invention. As used herein, the term "peptidomimetic" is intended to include peptide analogs which serve as appropriate substitutes for peptides in interactions with e.g., receptors and enzymes. The peptidomimetic must possess not only affinity, but also efficacy and substrate function. That is, a peptidomimetic exhibits functions of a peptide, without restriction of structure to amino acid constituents. Peptidomimetics, methods for their preparation and use are described in Morgan *et al.*, (1989) "Approaches to the discovery of non-peptide ligands for peptide receptors and peptidases". In *Annual Reports in Medicinal Chemistry* (Vinick, F.J., ed.) pp. 243-252, Academic Press, San Diego, CA. Many targeting moieties are known, and include, for example, asialoglycoproteins (see e.g., Wu, U.S. Patent 5,166,320) and other ligands which are transported into cells via receptor-mediated endocytosis (see below for further examples of targeting moieties which may be covalently or non-covalently bound to a target molecule).

In the methods of the invention, pain and/or inflammation in a subject is mitigated by administering an analgesic, sedative or anti-inflammatory therapeutic compound of the invention to the subject. The term "subject" is intended to include living organisms in which pain can occur. Examples of subjects include humans, apes, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. Administration of the compositions of the present invention to a subject to be treated can be carried out using known procedures, at dosages and for periods of time effective to alleviate pain in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compound to mitigate pain and inflammation in the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention (e.g., Va) is between 0.5 and 5000 mg/kg of body weight/per day, preferably between 50 and 1000 mg/kg/day, and still more preferably between 250 and 750 mg/kg/day. In an aqueous composition, preferred concentrations for the active compound

(i.e., the therapeutic compound that can mitigate pain) are between 5 and 500 mM, more preferably between 10 and 100 mM, and still more preferably between 20 and 50 mM.

According to the invention, therapeutic compounds are administered to a subject by a route which is effective for mitigating inflammation, effecting analgesia and/or effecting sedation. Suitable routes of administration include but are not limited to sublingual, oral, buccal, transdermal, nasal, subcutaneous, intraocular, intravenous, intramuscular and intraperitoneal (e.g., by injection). Preferred routes of administration are oral and transdermal. The therapeutic compounds can be administered with a pharmaceutically acceptable vehicle. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids, enzymes and other natural conditions which may inactivate the compound.

Therapeutic compounds of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB, they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties"), thus providing targeted drug delivery (see, e.g., Ranade *et al.*, 1989). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, 1988); antibodies (Bloeman *et al.*, 1995; Owais *et al.*, 1995); surfactant protein A receptor (Briscoe *et al.*, 1995). In a preferred embodiment, therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety.

Delivery and *in vivo* distribution can also be affected by alteration of an anionic group of compounds of the invention. For example, anionic groups such as phosphonate or carboxylate can be esterified to provide compounds with desirable pharmacokinetic, pharmacodynamic, biodistributive, or other properties. Exemplary compounds include IVI and pharmaceutically acceptable salts or esters thereof.

To administer a therapeutic compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a

material to prevent its inactivation. For example, a therapeutic compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes
5 (Strejan *et al.*, *J. Neuroimmunol.* (1984) 7, 27).

A therapeutic compound may also be administered parenterally (e.g., intramuscularly, intravenously, intraperitoneally, intraspinally, or intracerebrally). Dispersions can be prepared in glycerol, liquid polyethylene glycols, lactose, dextrose and mixtures thereof and in oils. Under ordinary conditions of storage and use, these
10 preparations may contain a preservative to prevent the growth of microorganisms. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable
15 under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, dextrose, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained,
20 for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some cases, it will be preferable to include isotonic agents, for
25 example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating a therapeutic
30 compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally,

dispersions are prepared by incorporating the therapeutic compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying
5 which yields a powder of the active ingredient (i.e., the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

A therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. A therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or
10 incorporated directly into the subject's diet. For oral therapeutic administration, a therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of therapeutic compound in the compositions and preparations may, of course, be varied. The amount of therapeutic compound in such therapeutically
15 useful compositions is such that a suitable dosage will be obtained.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound
20 calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of
25 pain and inflammation in subjects, or effecting sedation.

A therapeutic composition can be administered in time-release or depot form, to obtain sustained release of a therapeutic compound over time. A therapeutic compound of the invention can also be administered transdermally (e.g., by providing a therapeutic compound, with a suitable carrier, in patch form, or in an unguent or cream).

30 Active compounds are administered at a therapeutically effective dosage sufficient to mitigate pain and/or inflammation in a subject. The ability of a compound to mitigate

pain or inflammation can be evaluated in model systems that may be predictive of analgesia and anti-inflammation in human diseases, such as animal model systems known in the art (including, e.g., the preclinical acute pain writhing model in the mouse; and the formalin-sensitization model of tissue injury pain in the rat) or by *in vitro* methods, (including, e.g.,
5 the assays described above and below, *vide infra*). The ability of a compound to effect sedation can be evaluated in model systems that may be predictive of sedation of use in treatment of human diseases, such as animal model systems known in the art (including, e.g., the loss of the righting reflex in the mouse as described, *vide infra*) or by *in vitro* methods, (including, e.g., modulation of the activity of GABA_A receptors as described
10 above and below, *vide infra*).

It will be appreciated that the ability of a compound of the invention to mitigate pain and/or inflammation, in certain embodiments, be evaluated by observation of one or more symptoms or signs associated with pain and inflammation *in vivo*. Thus, for example, the ability of a compound to alleviate pain may be associated with an observable
15 improvement in a clinical manifestation of the underlying pain or inflammation related disease state or condition, or a slowing or delay in progression of symptoms of the condition. Thus, monitoring of clinical manifestations of disease can be useful in evaluating the analgesic, sedative and anti-inflammatory efficacy of a compound of the invention.

20 Treating or mitigating pain may involve effecting analgesia, effecting sedation, inhibiting or preventing inflammation, and/or ameliorating the manifestations or impact of pain inducing stimuli. Modulating a biological process such as the biological levels of cGMP or cAMP, or activity of soluble GCase, includes regulating increases and decreases in such activity, and inhibition, potentiation, agonism, or antagonism of the biological
25 process.

Methods of the invention are useful for treating pain and/or inflammation associated with any disease in which pain or inflammation occurs. Clinically, pain and inflammation can be associated with, but not limited to, tissue injury, post-operative tissue injury, nerve injury, post-herpetic neuralgia, phantom limb pain, diabetic neuropathy,
30 arthritis, dysmenorrhea, endometriosis, cancer, chemotherapy, myocardial infarction, cerebral vascular occlusion, or result from surgical procedures.

Certain compounds for use in the methods of the invention are commercially available, whereas others are novel (see hereinbelow and applicants' co-pending application USSN 09/267,379, filed March 15, 1999). Both types can be synthesized by standard techniques known in the art. In general, nitrate esters can be prepared from the
5 corresponding alcohol, oxirane or alkene by standard methods that include: nitration of alcohols and oxiranes, mixed aqueous/organic solvents using mixtures of nitric and sulfuric acid and/or their salts, with temperature control (see Yang *et al.*, 1996); nitration of alcohols and oxiranes in acetic anhydride using nitric acid or its salts with or without added acid catalyst, with temperature control (see, e.g., Louw, *et al.*, 1976); nitration of an
10 alcohol with a nitronium salt, e.g., a tetrafluoroborate; nitration of an alkene with thallium nitrate in an appropriate solvent (Ouellette *et al.*, 1976). Compounds of the present invention also can be prepared as described below.

The contents of all scientific publications and patent documents cited herein are hereby incorporated herein by reference in their entirety.

15 The following Examples further illustrate the present invention and are not intended to be limiting in any respect.

Examples

20 *Example 1: Characterization of guanylyl cyclase activation*

Activation of soluble guanylyl cyclase (GCase) by nitrates IIIm, IVa, IVb, IVd, IVe, IVf, IVg, IVj, Va, Vb, and GTN was assayed employing partially purified enzyme freshly prepared from the 105,000g supernatant fraction of rat aorta homogenates, using the radioimmunoassay method described by Bennett *et al.* (1992). Dose-response curves were
25 obtained for GCase activation by nitrates IVa, IVb, IVd, IVe, IVf, IVg, IVj, and GTN in the presence and absence of cysteine and dithiothreitol (DTT; both 2mM). In all cases, data were normalized to the maximal GTN response carried out in identical GCase preparations. Experimental incubations were performed at 37°C for 10 min. The data for IVd is summarized in Fig. 1. The GCase assay data show that IVd activates GCase, with a
30 submillimolar EC-50 (effective concentration for 50% of the maximal response) in the absence of any added thiol, in contrast to GTN, which requires added cysteine.

Compound IVd also activates GCase in the presence of DTT, in contrast to GTN, which, enigmatically, does not. Relative to GTN itself, a wide range of potency was observed for these novel nitrate esters. No activation of GCase by glycerol mononitrates was observed in this assay at the concentrations of nitrate employed.

5 Activation of GCase, *in vitro*, by some of these organic nitrates is via release of NO, since in the presence of cysteine, substantial NO is released at rates which are measurable amperometrically, using the method described by Artz and Thatcher (1998). By comparison, nitroglycerin, which is currently understood by others skilled in the art to act only as an NO-donor therapeutic agent, does not release NO at a rate measurable
10 amperometrically, in the absence nor the presence of cysteine. Relative rates for NO release, at 37°C, pH 7.4, in the presence of cysteine (2mM) from nitrates Vj, Vu, Vi, Vh, Vg, Vf, and Ve, (1mM) were 1.0, 1.0, 1.8, 0, 1.2, 0.5, 1.8, respectively. Thus modification of the structure of these organic nitrates can be used to control their NO-releasing ability and also to modulate GCase activity.

15 To test for potential differences in GCase activation by nitrates, the effects of IIIm, IVh, Va, Vb, and GTN were assayed in brain and vascular tissue. IVh had no effect on GCase activity in either rat aorta or rat hippocampus (Fig. 2). IIIm had greater efficacy to stimulate GCase activity compared to GTN in both rat aorta and rat hippocampus (Fig. 2). Vb was found to be equivalent to GTN in efficacy and potency for activation of GCase in
20 both rat aorta and rat hippocampus (Fig. 3). Va was found to have greater efficacy, but equal potency, to GTN in rat aorta (Fig. 3a). In contrast, Va had greater efficacy and greater potency to stimulate GCase in rat hippocampus (Fig. 3b). These data illustrate that nitrates have differential effects on GCase activation that are dependent on both structure of the compound and the tissue assayed for GCase activity, supporting the notion that
25 effects of nitrates elicited through GCase activation, such as analgesia and vasodilation, are separable and may be regulated in a tissue-specific and/or activity-specific manner, by appropriate choice of organic nitrate.

 As further examples of the potential for modulating potency, efficacy and tissue selectivity for activation of GCase, by choice of an appropriate organic nitrate, nitrates Va
30 and Vt were assayed in brain and vascular tissue. In the presence (+) and absence (-) of

1mM cysteine, the potency (EC-50 values) and efficacy (maximal activation) were measured for activation of GCase from rat hippocampus (Table 2).

Table 2	1mM cysteine	EC-50 (hipp.), M	EC-50 (aorta), M	maximal (hipp.), relative to GTN ^a	Maximal (aorta), Relative to GTN ^a
Va	+	1.4×10^{-4}	1.8×10^{-4}	5.1	2.2
Va	-	5.7×10^{-5}	1.8×10^{-4}	2.1	1.0
Vt	+	5.1×10^{-5}	6.5×10^{-5}	3.0	2.3
Vt	-	1.7×10^{-5}	1.8×10^{-6}	1.8	1.7
a) Normalized to GTN + 1mM cysteine maximal response.					

Example 2: Characterization of cyclic GMP accumulation

5 In order to extend the GCase data further, the effects of nitrates Va, IIIIm, Vb, Vc, and IVk on cyclic GMP accumulation in intact isolated rat aorta were examined (Figs. 4, 5). Thoracic aortic strips were prepared from male Sprague-Dawley rats (Charles-River, Canada) as described in McGuire *et al.* (1994) and Stewart *et al.* (1989). Tissues were contracted submaximally with phenylephrine (0.1μM) and exposed to various

10 concentrations of drug for 1 min. Cyclic GMP accumulation was determined using the radioimmunoassay method described by Bennett *et al.* (1992). At concentrations of 1 μM and 10 μM, GTN and IVk significantly increased cGMP accumulation (Fig. 5). At a concentration of 1 μM, Va, IIIIm, Vb, and Vc did not significantly increase cyclic GMP accumulation (Figs. 4a, 5a). At a concentration of 10 μM, Va, Vb, and IVk significantly

15 increased cyclic GMP accumulation whereas IIIIm and Vc did not (Figs. 4b, 5b).

Sections of rat hippocampus (400 μm) were prepared and incubated in oxygenated Krebs solution at 37°C. After a 60-min equilibration period, the brain slices were stimulated with different concentrations of Va or GTN for 3-min. Cyclic GMP accumulation was determined as described above for aortic strips. Figure 6 shows that Va

20 causes a concentration-dependent increase in the tissue levels of cGMP in rat hippocampal brain slices *in vitro*, and that at high concentration (100 μM) Va is more effective than GTN in elevating cGMP levels in hippocampal brain slices *in vitro*. These data are in very

good agreement with the differential effects of Va and GTN on hippocampal GCase activity shown in Figure 3b.

Example 3: Characterization of relaxation of isolated blood vessels

5 In order to extend the GCase data, the relaxing effects of nitrates III_m, IV_c, IV_d, IV_f, IV_g, IV_h, IV_k, Va, Vb, and Vc on rat aortic tissue were examined. Thoracic aortic strips were prepared from male Sprague-Dawley rats (Charles-River, Canada) as described in McGuire *et al.* (1994), and Stewart *et al.* (1989). Tissues were contracted submaximally with phenylephrine (0.1 μM) and exposed to various concentrations of nitrate esters to
10 obtain concentration-response curves. In this intact tissue assay, all of the nitrates were observed to cause relaxation of the tissue with a maximal relaxant response equal to that obtained with GTN. However, the compounds differed in potency, with EC-50 values of 7.87 nM, 94.3 nM, 6.59 μM, 25.2 μM, 11.0 μM, and 0.203 μM, for GTN and compounds Va, IV_d, IV_g, IV_f, and IV_c, respectively (Fig. 7). In another series of experiments, the EC-
15 50 values for relaxation were 0.61 nM, 3.19 nM, 8.40 nM, 0.153 μM, 0.437 μM and 6.89 μM for GTN, IV_k, Vb, III_m, Vc, and IV_h, respectively (Fig. 8). Compounds IV_d and IV_c were tested for their ability to cause vascular relaxation in tissues that had been made tolerant to the relaxant effect of GTN. GTN tolerance was induced by incubating tissues with a high concentration of GTN (0.5 mM GTN for 30 min). Under these conditions, the
20 maximal relaxant effects of IV_d (Fig. 12a) and IV_c (Fig. 12b) were not significantly different to that of untreated tissue. The EC-50 for relaxation was increased approximately threefold, but the difference was not statistically significant.

Example 4: Characterization of blood pressure changes in the whole animal

25 To test for differential effects of nitrates on blood pressure responses, Va and GTN were injected into rats in which the abdominal aorta was cannulated for blood pressure recording. In the first experiment, Va and GTN were injected subcutaneously at a dose of 400 μmol/kg body weight into conscious, freely moving animals. GTN caused a small and transient decrease in blood pressure in these animals, whereas Va had no discernable effect
30 on arterial blood pressure (Fig. 9). Va and GTN were subsequently tested in anesthetized rats in which the abdominal vena cava was also cannulated to allow for bolus intravenous

injection of drugs. In this preparation, GTN caused a substantial and dose-dependent decrease in arterial blood pressure. In contrast, Va at equal doses had very modest effects on blood pressure at doses lower than 2 $\mu\text{mol/kg}$ body weight (Fig. 10). These data are in very good agreement with the results obtained for these two agents using the isolated
5 blood vessel preparation.

The plasma levels of nitrates Vb and Vc (the denitrated metabolite of Vb) were measured to gain insight into the handling of these molecules in the body. Cannulas were placed in the abdominal aorta for blood sampling. After a two-day recovery period, a single subcutaneous dose of Vb (200 $\mu\text{mol/kg}$) was administered and blood samples
10 collected over a period of six hours. Samples were centrifuged, the plasma collected, and the concentration of Vb and Vc determined by gas-liquid chromatography by the method of McDonald and Bennett (1990). The data obtained for Vb and Vc indicate that nitrates achieve maximal plasma levels within 30 minutes after subcutaneous injection, and thereafter decline at a steady rate (Fig. 11). These data suggest that nitrates have excellent
15 bioavailability after subcutaneous injection.

Example 5: Characterization of the analgesic effects of novel organic nitrates in a model of acute pain

Injection of dilute acetic acid solutions into the peritoneum of a mouse induces
20 writhing movements that can be quantified. We adopted the methodology described by Bak *et al.* (1998) to test for analgesic effects of organic nitrates in this mouse model. Each mouse was given an intraperitoneal injection of 0.5 mL of a 0.6% solution of acetic acid in distilled water. After a 5 minute delay, the number of writhing movements was counted over a 10 minute period. To test the efficacy of novel organic nitrates in this model, drugs
25 were administered at doses of 100-500 mg/kg (given by subcutaneous injection) 15 minutes before the intraperitoneal injection of acetic acid. In this model of acute pain, Vm induced a significant, dose-dependent analgesic effect, manifested as a decrease in the number of writhes per 10 minute period after intraperitoneal injection of dilute acetic acid (Figure 13a). Va was also able to act as an analgesic (decreased writhing) in this experimental
30 model when administered at a dose of 500 mg/kg subcutaneously (Figure 13b).

Example 6: Characterization of the analgesic effects of novel organic nitrates in a model of hyperalgesia/allodynia

Under light halothane anesthesia, male Sprague-Dawley rats were injected subcutaneously with 0.05 mL of 5% formalin into the dorsal surface of one hind paw as described in Malmberg and Yaksh (*Anesthesiology* (1993) 79, 270-281). The number of spontaneous paw flinches was determined in 1 minute blocks at 5 minute intervals for 60 minutes. Formalin injection in the paw produces two distinct phases of pain; an acute phase occurring within the first 5-10 minutes, and a delayed phase that develops between 15-30 minutes after formalin injection. The acute phase of the pain response to formalin is caused by activation of peripheral nociceptive sensory afferents (C-fibres) by the peripheral stimulus. The delayed pain response is considered to be a hyperalgesia/allodynia caused by a combination of sensitization of peripheral sensory afferents and sensitization of synaptic connections in the spinal cord. The formalin test in the rat is considered to be an appropriate model for tissue injury pain occurring in humans (Tjolsen *et al.*, *Pain* (1992) 51, 5-17; Yaksh, *TIPS* (1999) 20, 329-337). In this experimental model of inflammatory tissue injury pain, Vm (500 mg/kg) significantly reduced both phases of the pain response to formalin injection (Fig. 14a,b).

Example 7: Synthesis of IIIe

To acetic anhydride (3 mL) was added gradually, with stirring, 70% nitric acid (0.26 mL), while keeping the temperature between 20-30° by external cooling. With continuous vigorous stirring the mixture was cooled to -30-35° and 2',3'-dideoxy-3-thiocyctosine (0.25 g) was added. After 10 min at -35°, the reaction mixture was heated up to -20° and then stirred at -20-10° for 15 min and 10 min at 0°. The resulting reaction mixture was poured into ice-water, stirred for 1 h, then NaHCO₃ was added by portions until CO₂ evolution ceased. The water solution was extracted with 3x20 mL of ethyl acetate. Combined extracts were dried (MgSO₄) and concentrated. 0.38 g of slightly yellowish oil was obtained. The oil crystallized in a day and was recrystallized from CHCl₃. Yield 52%. Conversion to the nitrate was evidenced by the significant downfield shift of the C5' proton multiplet from δ 3.6 to 4.85 ppm.

Example 8: Synthesis of nitrate IIIf

0.26 mL (4.15 mmol) conc. HNO_3 was added to 2 mL acetic anhydride such that the temperature did not exceed 25 – 30 °C. The mixture was cooled at 0 – 5 °C and 0.3 g
5 (1.88 mmol) of 5-(1,2-dihydroxyethyl)-4-methylthiazole was added in several portions, the temperature being kept below 5 °C. The reaction mixture was stirred at 0 – 5 °C for 45 min and then 0.45 mL water was added. The mixture was stirred for 30 min and then rotary evaporated. The residue was neutralized by adding 5 mL of saturated NaHCO_3 solution and the organic product was extracted with ethyl acetate. The organic layer was
10 concentrated and the dinitrate IIIf was purified through column chromatography (silica gel/ ethyl acetate eluant). A slightly yellow solid was obtained. Yield: 0.150 g (32 %).

Example 9: Synthesis of nitrate IIIi

Nitrate IIIi was obtained by two routes. Route I proceeded from the elimination
15 reaction of IIIm in basic solution. Route II proceeded from nitration of *trans*-3-bromo-4-hydroxytetrahydrothiophene-1,1-dioxide, yielding nitrate IIIn, followed by reaction with a weak base, e.g., sodium thiocyanate in 2-butanone. Purification may be achieved with silica flash column chromatography using 1:1 hexane:ethyl acetate as eluant.

Example 10: Synthesis of nitrate IIIj

1,4-Dibromo-2,3-butanediol may be nitrated: (a) using a nitration mixture prepared from HNO_3 and H_2SO_4 over 2 days; or (b) using acetyl nitrate reacting for 2 hours. Work-up requires quenching of the reaction mixture in ice-water for an hour, extraction, drying, and evaporation. Successful purification of the title compound by silica gel column
25 chromatography is achieved on a 25 g scale using a mixture of 70% hexane and 30% CH_2Cl_2 as eluent.

Example 11: Synthesis of nitrate Ve

4-Methylbenzenethiol was obtained by adaptation of literature procedures from p-toluidine (J.-P. Morizur, *Bull. Soc. Chim. Fr.* (1964) 1338-1342; Bourgeois, *Recl. Trav. Chim. Pays-Bas* (1899) 18, 445-450). p-Toluidine hydrochloride (14.2 g, 0.098 mole) was diazotised
30

at 5°C with concentrated hydrochloric acid (16.5 mL) and sodium nitrite (7.2 g, 0.104 mole) in water (12 mL). The solution of diazonium salt was added over 1.5 h to a solution of ethyl xanthate (24 g, 0.149 mole) in water (30 mL) at 45-50°C. The mixture was kept at this temperature, under stirring, for a further 1 h. The xanthate ester was separated as a maroon oil, washed with 50 mL 10 % NaOH and with water to neutral pH and dried over MgSO₄ (20g of crude product). The crude xanthate was dissolved in 60 mL absolute ethanol and to this solution 20 g KOH (pellets) were added in portions. The reaction mixture was refluxed under stirring and Ar for 8 h, then concentrated under vacuum. The concentrate was taken up in 50 mL H₂O and extracted with 3x100 mL diethyl ether. The aqueous layer was acidified with a 6N H₂SO₄ solution and extracted with 3x100 mL CH₂Cl₂. The combined extracts were washed with water, dried over MgSO₄, evaporated and flash columned on silica gel, eluant hexanes:ethyl acetate=9:1, giving 10 g (81.56 %) of 4-methylbenzenethiol. ¹H-NMR(CDCl₃, 300MHz): 7.18-7.24 (m, arom 2H), 7.04-7.11 (d, arom 2H, J 7.93), 3.41(s, 1H), 2.32(s, 3H). ¹³C-NMR(75.48 MHz): 21.34, 128.95, 130.24, 130.29, 136.05.

The dinitrate IVd (9.67 mmoles) was dissolved in 10 mL distilled water and the solution kept under Ar for 30 minutes. To this solution, a solution of 0.8 g (6.46 mmoles) of 4-methylbenzenethiol and 7 mL 1M NaOH was added dropwise. The resulting emulsion was stirred for 15 min and then extracted with 3x20 mL CH₂Cl₂. The combined organic extracts were washed with H₂O, dried over MgSO₄ and concentrated under vacuum. The remaining oil was purified by flash column chromatography on silica gel, eluant hexanes: ethyl acetate=9:1, giving the product Ve (1.097 g, 52.22 %). ¹H-NMR(CDCl₃, 300MHz): 7.44-7.51 (m, arom 2H), 7.17-7.24 (d, arom 2H, J 7.91), 5.47-5.59 (m, 1H), 4.83-4.93 (dd, 1H, J 12.81, 2.78), 4.57-4.67 (dd, 1H, J 12.82, 5.71), 3.02-3.12 (dd, 1H, J 14.48, 6.01), 2.9-2.99 (dd, 1H, J 14.47, 7.72), 2.38 (s, 3H). ¹³C-NMR(75.48 MHz): 21.53, 36.78, 69.82, 77.68, 130.52, 130.62, 132.55, 139.23.

Example 12: Synthesis of nitrate IIIIm

3,4-Epoxytetrahydrothiophene-1,1-dioxide (250 mg, 1.9 mmol) was refluxed for 24 h in 10 mL of water and 25mg of toluenesulfonic acid. After the first 6 h, another 25 mg of the acid was added. The reaction was monitored by thin layer chromatography (TLC) (5%

methanol in dichloromethane). Purification was by Si flash column chromatography using 5% methanol/ CH_2Cl_2 as eluent to afford 200 mg of diol. The diol was nitrated in a cooled solution of conc. sulfuric acid (2 mol eq.), nitric acid (70%, 2 mol eq.) in an ice bath. The temperature was maintained as close to 0°C as possible. The ice bath was removed and the mixture was allowed to stir for 1 hour (reaction was monitored by TLC, 100% CH_2Cl_2 eluent). The acid layer was removed and the organic layer washed with: (i) water; (ii) 10% sodium carbonate; (iii) 10% urea; (iv) water. Drying over sodium sulfate, filtration and concentration, yielded crude product which was purified by flash column chromatography, with dichloromethane as eluent. An alternative route involves direct nitration of 3,4-epoxytetrahydrothiophene-1,1-dioxide in a similar nitration mixture.

Example 13: Synthesis of nitrate IVk

1.17 mL (18.2 mmol) concentrated HNO_3 was added, under stirring and cooling ($0 - 5^\circ\text{C}$), to 1 mL (18.2 mmol) concentrated H_2SO_4 and then 2 g (14 mmol) of 4-methyl-5-(2-hydroxyethyl)thiazole was added dropwise into the nitration mixture, the temperature being kept under 10°C . The mixture was stirred for 3 hours at room temperature, diluted with 10 mL of water and neutralized with solid NaHCO_3 . The organic product was extracted with ethyl acetate and purified by column chromatography (silica gel/ ethyl acetate eluant) to produce a colorless oily product. Yield: 1.18 g (45%).

Example 14: Synthesis of nitrate IVi

0.03 g (0.035 mL) of allyl cyanide was added to a stirred suspension of 0.22 g (0.5 mmol) of $\text{Ti}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$ in 2 mL of pentane. After 20 min of vigorous stirring the pentane solution was decanted and evaporated to dryness. After evaporation the residual oil (0.44 g) was columned (CH_2Cl_2 , Rf 0.64 (CH_2Cl_2)). Clean oil immediately crystallized during attempt to dissolve it in CDCl_3 . Yield 0.065 g (76%). The structure of IVi was confirmed by X-ray analysis. IR (film): 1297.03, 1678.91, 2258.91 (CN). Mass spec. m/z (Cl^+ fragment, %): 191.9 (M+H, 2.44), 129.0 (16.41), 81.9 (100). Calculated for $\text{C}_4\text{H}_5\text{N}_3\text{O}_6$ 191.02.

Example 15: Synthesis of nitrate IVm

0.9 g (0.75 mL, 4.92 mmol) of allylphenyl sulfone was added dropwise to a stirred suspension of 2.43 g (5.47 mmol) of $\text{Ti}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$ in 10 mL of pentane. The resulting mixture was stirred overnight. The pentane solution was decanted. 2x10 mL of methanol were added to the reaction mixture, stirred for 10 minutes and extracts were added to the pentane solution. The combined extracts evaporated to dryness and purified by silica flash column chromatography using CH_2Cl_2 as eluant. Yield 0.08 g (15%). IR (KBr): 1152.39, 1290.91, 1273.12, 1353.83, 1646.08. Mass spec. m/z (CI^+ fragment, %): 307.0 (M+1, 66.5), 244.0 (100%). Calculated for $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_8\text{S}$ 306.02.

Example 16: Synthesis of nitrate Va

2.2 g (7.3 mmol) of nitrate IVd was dissolved in 5 g of cold H_2O_2 (30%, 0°C) and then 1 g of 10 % H_2SO_4 was added. The mixture was stirred at 0-5 °C until a white oil separated (ca. 30-60 min). The aqueous layer was discarded and the oil was dissolved in dichloromethane, washed successively with water, then NaHCO_3 solution and finally water. The organic solution was dried over MgSO_4 . Removal of the solvent produced 1.3 g of the crude product which was purified by column chromatography (Silicagel, CH_2Cl_2 /hexanes : 70/30). Yield: 0.650 g (45 %).

Example 17: Synthesis of nitrate Vc

3 g (8.88 mmol) of 1,4-dibromo-2,3-dinitrobutanediol and 2.81 g (18 mmol) of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ were dissolved in the mixture of 100 mL of methanol and 45 mL of H_2O . The resulting solution was heated during 4 days at 40-45°. After this time the reaction mixture was partially evaporated to reduce the volume of solvents. The resulting mixture was extracted 4x50 mL of ethyl ether. The extracts were combined, washed (H_2O), dried (MgSO_4) and evaporated to minimum. Column chromatography afforded the title compound in 10% yield, separated from Vb the major product.

Example 18: Synthesis of nitrate Vy

The title compound was synthesised by the reaction of IVd with 4-methyl-5-thiazole ethanthiol, in a similar procedure to that used in Example 11. The crude product

was purified by column chromatography (Silicagel, ethyl acetate eluant) to give product (50 mg, 27.32 %). ¹H-NMR(CDCl₃, 300MHz): 8.22 (s, 1H), 5.49-5.6 (m, 1H), 4.9-5.00 (dd, 1H), 4.64-4.78 (dd, 1H), 3.14-3.22 (t, 2H), 2.89-3.07 (m, 4H), 2.45 (s, 3H). Mass spec., m/z (EI⁺, fragment, %): 355.0. Calculated for C₉H₁₃N₃O₆S₃: 355.0. The 4-Methyl-5-thiazole precursor was obtained from 4-methyl-5-thiazole ethanol, thiourea and hydrobromic acid by adaptation of literature procedures (R.L. Frank, P. V. Smith, *J. Am. Chem. Soc.* (1946) 68, 2103-2104). A mixture of 2 g 4-methyl-5-thiazole ethanol (13.965 mmol), 1.063 g (13.965 mmol) thiourea and 9.45 g (56 mmol) hydrogen bromide as 48 % hydrobromic acid was refluxed for 7 h with stirring, under Ar. A solution of 2.24 g (56 mmol) of NaOH in 20 mL of water was then added and the mixture was refluxed without stirring for 2 h. The layers were separated, and the acidified aqueous layer was extracted with three 30 mL portions of CH₂Cl₂. The extracts and original organic layer were combined, dried over MgSO₄ and concentrated under vacuum to afford 1.9 g crude product which was purified by column chromatography using ethyl acetate as eluent (Yield 1.6 g, 75%). ¹H-NMR(CDCl₃, 300MHz): 8.5 (s, 1H), 2.94-3.02 (t, 2H), 2.62-2.72 (q, 2H), 2.33 (s, 3H), 1.38-1.45 (t, 1H). ¹³C-NMR(75.48 MHz): 15.41, 26.41, 31.51, 39.72, 129.36, 149.90

Example 19: Synthesis of nitrate IIIk and IIIl

Synthesis from dinitrate IIIj proceeded by refluxing with sodium or potassium thiocyanate (2 eq.) in 2-butanone for 8 h. After cooling, a precipitate was removed by filtration and the filtrate was concentrated. Nitrates IIIk and IIIl were separated by silica flash column chromatography with hexane/dichloromethane as eluent.

Example 20: Synthesis of nitrate IIIaj.

A mixture of 8.56 g (63 mmol) of 4-bromo-1-butene, 32 mL of 88%-formic acid and 11 mL of 30%-H₂O₂ was stirred for 2 h at 50-56° (M. Pailer, *Monash. Chem.*, 1971, 102, 1048-1054). The resulting solution was stirred overnight, before removal of H₂O and HCOOH by distillation. To the residue was added 50 mL of a methanolic solution of HCl, and the mixture refluxed for 1 h. Volatiles were removed by rotary evaporation to yield 3,4-dihydroxy-1-butyl bromide, 8 g of which was nitrated in a mixture of 6.5 mL of conc.

H₂SO₄ and 7.3 ml of 70%-HNO₃ in the usual manner. The resulting greenish solution was added with care to ice-water, and the layers separated. The aqueous portion was extracted with CH₂Cl₂ (3x30 mL). Combined organic layers were washed twice with 10 mL of 10% NaHCO₃, then water, dried over MgSO₄, volatiles evaporated, and the residue purified by chromatography to IIIaa (Silica gel, CH₂Cl₂/hexanes : 70/30). Yield: 3.85 g (32 %). ¹H-NMR(CDCl₃, 300 MHz): 5.48-5.57 (1H, m), 4.79-4.88 (1H, dd, J 13.08, 3.05), 4.44-4.54 (1H, dd, J 13.08, 5.48), 3.38-3.55 (2H, m), 2.12-2.41 (2H, superposition of 2 multiplets). ¹³C-NMR: (CDCl₃, 75.48 MHz): 76.92, 70.74, 31.76, 27.02.

To a solution of IIIaa 1.8 g (6.98 mmol) in 30 ml of CH₃OH and 10 ml of H₂O, was added 1.1 g (6.98 mmol) of Na₂S₂O₃, and the resulting suspension was stirred rigorously for 2.5 days giving a yellowish turbid solution. The solution was evaporated to dryness giving 2.5 g of the crude product, IIIai, as a yellowish solid. NMR revealed the desired product in 96% purity. ¹³C-NMR: (CD₃OD, 100.61MHz): 79.60, 72.88, 31.12, 29.91 This product (0.43 g, 1.37 mmol) was dissolved in 10 mL of distilled water and the emulsion of 0.23 g (1.28 mmol) of the ethyl ester of thiosalicylic acid in 1.3 mL of 1 M NaOH was added. The resulting solution immediately became turbid and was stirred for 3 min, then extracted with ethyl acetate (4x15 mL). The resulting extracts were washed with H₂O, dried (MgSO₄), and concentrated by evaporation. The residue was flash columned on silica gel, eluant hexane:ethyl acetate=9:1 (R_f 0.23), giving 0.27 g (55%) of IIIaj. ¹H-NMR(CDCl₃, 300 MHz): 8.06-8.12 (1H, dd, J 8.12, 0.36), 7.99-8.04 (1H, dd, J 7.8, 1.15), 7.51-7.59 (1H, m, J 7.24, 1.44), 7.21-7.29 (1H, m, J 7.35, 0.54), 5.40-5.49 (1H, m), 4.70-4.78 (1H, dd, J 13.04, 2.95), 4.33-4.45 (3H, m, superposition of dd (J 13.08, 6.01) of 1H from CH₂-ONO₂ and quart. from O-CH₂-CH₃), 2.66-2.87 (2H, m), 2.06-2.15 (2H, quart., J 6.92), 1.35-1.43 (3H, t, J 7.14). ¹³C-NMR: (CDCl₃, 75.48 MHz): 166.21, 140.41, 132.78, 131.46, 127.63, 125.48, 77.25, 71.03, 61.42, 32.51, 28.29, 14.17. Mass spec. m/z (EI⁺, fragment, %): 392.3 (M⁺, 3.69), 153 (100). Calculated for C₁₃H₁₆N₂O₈S₂ 392.03.

Example 21: Chiral synthesis of nitrate IVd

A 50 mL round-bottom flask, equipped with a magnetic stirrer, was charged with 10 mL of *tert*-butyl alcohol, 10 mL of water, and 2.8 g of a catalyst (AD-mix-β, Aldrich: K.B.Sharpless, W.Amberg, Y.L.Bennani, G.A.Crispino, J.Hartung, K.-S.Jeong, H.-

L.Kwong, K.Morikawa, Z.-M.Wang, D.Xu, X.-L.Zhang, *J.Org.Chem.* (1992) 57, 2768-2771). Stirring at room temperature produced two clear phases; the lower aqueous phase appears bright yellow. The mixture was cooled to 4° and 0.2 mL (2 mmol) of allylbromide was added at once, and the heterogeneous slurry was stirred vigorously at 4-5° for 2.5 h (monitoring by TLC hexane:methanol=1:9). While the mixture was stirred at 0°C, solid sodium sulfite.(3 g) was added and the mixture was allowed to warm to room temperature and stirred for 1 h. Then 20 mL of ethyl acetate was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with ethyl acetate. The combined organic extracts were dried (MgSO₄), concentrated *in vacuo* and purified by flash chromatography on silica (hexane:methanol=1:9, R_f 0.5), to yield chiral 1-bromo-2,3-propanediol. Yield 0.2 g (55.5%). Optical rotation: minus. Nitration to 1-bromo-2,3-dinitroxypropane was achieved using reaction in HNO₃/H₂SO₄ (K. Yang, J.D. Artz, J. Lock, C. Sanchez, B.M. Bennett, A.B. Fraser, G.R.J. Thatcher, *J.Chem.Soc., Perkin Trans.* (1996) 1, 1073-1075) and the product was purified by flash chromatography on silica gel (hexane:CH₂Cl₂ = 2:3, R_f 0.5). Yield 55.5%. Optical rotation: minus. The dinitrate IVd was synthesised from chiral 1-bromo-2,3-propanediol by our usual procedure (K. Yang, J.D. Artz, J. Lock, C. Sanchez, B.M. Bennett, A.B. Fraser, G.R.J. Thatcher, *J.Chem.Soc., Perkin Trans.* (1996) 1, 1073-1075). and purified by flash chromatography on silica (ethyl acetate:methanol=9:1). Yield 2.27%. Optical rotation: plus.

Example 22: Chiral synthesis of nitrate Vm

The title compound was synthesised by the reaction of the stereochemically resolved, chiral dinitrate IVd (1g, 3.3 mmoles) with ethyl thiosalicylate (0.6 g, 3.3 mmoles) in the presence of 3.3 mL of 1M NaOH.. The crude product was purified by column chromatography (Silicagel, hexanes/EtOAc: 1/10). ¹H-NMR(CDCl₃, 300MHz):8.04-8.11 (m, arom 2H), 7.55-7.62 (m, arom 1H), 7.30-7.34 (m, arom 1H), 5.43-5.54 (m, 1H), 4.88-4.97 (dd, 1H, J 12.95, 2.79), 4.62-4.71 (dd, 1H, J 12.94, 5.35), 4.45-4.39 (q, 2H, J 7.12), 2.92-3.08 (m, 2H), 1.39-1.47(t, 3H, J 7.13). ¹³C-NMR(75.48 MHz): 14.17, 26.23, 35.95, 61.55, 69.54, 77.24, 125.56, 125.92, 127.91, 131.52, 132.93, 139.56, 166.189. Mass spec., m/z (EI⁺, fragment, %): 378.18 (1.04), 152.9 (100) calculated for C₁₂H₁₄N₂O₈S₂: 378.02

Example 23: Synthesis of Vx

The title compound was synthesised by the reaction of IVd (0.345g, 1.15 mmol) with captopril methyl ester (0.2 g, 0.865 mmol) in the presence of 1 mL of 1M NaOH. The crude product was purified by column chromatography (Silicagel, ethyl acetate) to afford 0.1 g (18.03 %) of unsymmetrical disulphide. ¹H-NMR (CDCl₃, 300MHz): 5.49-5.61 (m, 1H), 4.88-4.90 (m, 1H), 4.62-4.74 (m, 1H), 4.40-4.55 (m, 1H), 3.59-3.78 (m, superpos. 5H), 2.69-3.21 (m, 5H), 2.14-2.29 (m, 1H), 1.90-2.12 (m, 3H), 1.22-1.28 (d, 3H). ¹³C-NMR(75.48 MHz): a mixture of enantiomers can be seen: 17.39, 25.20, 29.40, 36.41, 36.51, 38.55, 41.94, 42.19, 47.32, 52.56, 59.12, 69.90, 70.29, 77.89, 78.00, 173.01, 173.48.

Example 24: Synthesis of nitrate Vq

The title compound was synthesized by the reaction of IVd (2.4 g, 8 mmol) with 4,4'-thiobisbenzenethiol (0.5 g, 2 mmol) in the presence of 4.4 mL of 1M NaOH. The crude product was purified by column chromatography (silica gel, hexanes/CH₂Cl₂: 3/7) to afford 0.28 g (21.82 %) disulfide. ¹H-NMR(CDCl₃, 300MHz): 7.2-7.6 (m, arom 8H), 5.42- 5.56 (m, 2H), 4.82-4.95 (dd, 2H), 4.55- 4.67 (dd, 2H), 2.93-3.15 (m, 4H).

Example 25: Synthesis of nitrate Vr

The title compound was synthesised by the reaction of IVd (0.5 g, 1.58 mmol) with ethyl 2-mercapto-3-(3',4'-methylenedioxy-phenyl) propenoate (0.2 g, 0.8 mmol). The crude product was purified by column chromatography (Silicagel, hexanes/CH₂Cl₂: 3/7) to give Vr (0.1 g, 28.09 %). ¹H-NMR(CDCl₃, 300MHz): 7.97 (s, 1H), 7.48-7.51 (d, 1H), 7.17-7.22 (m, 1H), 6.84-6.89 (d, 1H), 6.05 (s, 2H), 5.48-5.58 (m, 1H), 4.81-4.89 (dd, 1H), 4.53-4.61 (dd, 1H), 4.25-4.4 (m, 2H), 3.05-3.10 (m, 2H), 1.35-1.45 (t, 3H). ¹³C-NMR(75.48 MHz): 14.17, 36.27, 62.15, 69.71, 76.57, 101.77, 108.27, 110.31, 125.20, 127.55, 127.87, 146.87, 147.811, 149.76, 165.89.

Ethyl 2-mercapto-3-(3',4'-methylenedioxy-phenyl) propenoate was obtained by the following route:

(1) Ethylisothiocyanate (10 g, 0.115 moles), 7.9 g (0.086 moles) mercaptoacetic acid and 5 mL pyridine in C₆H₆ was refluxed, cooled and filtered yielding 3-ethylrhodanine, which was used in the next step without any further purification. Reaction of 3-ethylrhodanine

with piperonal in the presence of sodium acetate was performed in CH_3OH under refluxing for 1 h. The yellow precipitate obtained after cooling and filtration, was washed several times with CH_3OH on the filter and crystallized from CH_3OH to give 3-ethyl-5-piperilidenerodanine. $^1\text{H-NMR}(\text{DMSO-}d_6, 300\text{MHz})$: 7.72 (s, 1H), 7.1-7.3 (m, 3H), 6.14 (s, 2H), 3.95-4.15 (q, 2H), 1.15-1.20 (t, 3H). $^{13}\text{C-NMR}(75.48\text{ MHz})$: 12.76, 40.28, 103.07, 110.21, 110.47, 120.68, 127.89, 128.07, 134.01, 149.23, 150.74, 167.57, 193.68.

(2) 3-Ethyl-5-piperilidene-rodanine (1g, 3.4 mmol) was added to a stirred solution of 0.16 g (6.8 mmol) in 8 mL abs. ethanol and the mixture was refluxed for 30 min. To the solution cooled to room temperature 5 mL H_2O were added and the mixture was hydrolyzed with 10% HCl and extracted with ether. The ether phase was separated, dried (MgSO_4) and evaporated to an oil. Since the investigation by TLC of the crude reaction mixture indicated the presence of piperonal, the reaction mixture was dissolved in CH_2Cl_2 and the obtained solution was washed with 1M NaOH. The aqueous phase was extracted twice with CH_2Cl_2 and then neutralized with dilute HCl. Free thiol was extracted with ethyl ether. After concentration, the product was purified by column chromatography eluting with hexane/ethyl ether:8/2. Yield 0.4 g (62.4 %). $^1\text{H-NMR}(\text{CDCl}_3, 300\text{MHz})$: 7.69 (s, 1H), 7.725-7.29 (d, 1H, J 1.46), 7.1-7.18 (m, 1H), 6.85-6.9 (d, 1H, J 8.13), 6.01 (s, 2H), 4.75 (s, 1H), 4.28-4.38 (q, 2H, J 7.12), 1.35-1.42 (t, 3H, J 7.13). $^{13}\text{C-NMR}(75.48\text{ MHz})$: 14.20, 62.5, 101.41, 108.37, 109.43, 121.06, 125.45, 129.20, 134.68, 147.80, 148.05, 165.43.

Example 26: Synthesis of nitrate IVs

The title compound was synthesised by the reaction of IVb with dithiothreitol in CH_3OH and was isolated as an oil in 15-20% yield (CAUTION: stench). $^1\text{H-NMR}(\text{CDCl}_3, 300\text{ MHz})$: 5.23-5.32 (1H, m), 4.87 (1H, dd, J 12.82, 3.22), 4.68 (1H, dd, J 12.83, 6.09), 2.77-2.94 (2H, m), 1.66 (1H, t, J 9.07). $^{13}\text{C-NMR}(\text{CDCl}_3, 75.48\text{ MHz})$: 79.39, 69.30, 23.68.

Example 27: Synthesis of nitrate IVt

The title compound was synthesised by the reaction of nitrate IVs with acetyl chloride in CHCl_3 . Isolated yield 50%. $^1\text{H-NMR}(\text{CDCl}_3, 300\text{ MHz})$: 5.29-5.38 (1H, m),

4.76 (1H, dd, J 12.94, 3.11), 4.55 (1H, dd, J 12.94, 6.37), 3.30 (1H, dd, J 14.06, 5.98), 3.13 (1H, dd, J 14.61, 6.35). ^{13}C -NMR: (CDCl_3 , 75.48 MHz): 194.10, 77.00, 69.79, 30.42, 27.78. Mass spec. m/z (EI^+ , fragment, %): 240.0 (M^+ , 1.17), 193.9 ($\text{M}-\text{NO}_2$, 10.86), 148.8 (100). Calculated for $\text{C}_5\text{H}_8\text{N}_2\text{O}_7\text{S}$ 240.01.

5

Example 28: Synthesis of nitrate IIIw

Diethyl 1-chloro-2-trimethylsiloxypropylphosphonate was obtained by adaptation of literature methods (T.Azuhata, Y.Okamoto, *Synthesis* (1983) 916-917). This phosphonate was quantitatively converted to diethyl 1-chloro-2-hydroxypropylphosphonate using CH_3OH . After stirring for 15 min the resulting reaction mixture was evaporated to a minimum and subjected to nitration with a mixture of HNO_3 and H_2SO_4 . Work-up and flash column chromatography on silica (ethyl acetate eluant) yielded pure product in 25% yield. ^{31}P (CDCl_3 , 162 MHz): 24.60. ^1H (CDCl_3 , 300 MHz): 5.31-5.45 (m, 1H), 3.92-4.08 (m, 4H), 3.63-3.81 (m, 2H), 2.03-2.30 (m, 2H), 1.16-1.24 (superposition of 2 t, 6H, J 7). ^{13}C (CDCl_3 , 75 MHz): 76.83, 62.15 (d, J 6.37), 43.77 (d, J 8.95), 27.08 (d, J 142.00), 15.99 (d, J 5.88).

15

Example 29: Synthesis of nitrate IIIx

Treatment of IIIw with 1 mole of Me_3SiBr for 1 h with subsequent addition of CH_3OH provided the monodealkylated phosphonic acid in high purity. Transformation of the free acid to its sodium salt IIIx was achieved using the cation-exchange resin Amberlite IR-122- Na^+ form. ^{31}P (CD_3OD , 122 MHz): 17.62. ^1H (CD_3OD , 300 MHz): 5.38-5.63 (m, 1H), 3.75-4.25 (superposition of 2 m, 4H), 1.88-2.20 (m, 2H), 1.12-1.28 (t, 3H). ^{13}C (CD_3OD , 75 MHz): 81.14, 61.17 (d, J 5.41), 45.56 (d, J 5.94), 29.35 (d, J 131.74), 17.00 (d, J 6.75).

20

25

Example 30:

The effect of IVk was tested in *Xenopus* oocytes expressing human recombinant GABA_A receptors by the two-electrode voltage clamp technique as described in Reynolds and Maitra (*European Journal of Pharmacology* (1996) 314, 151-156). The control GABA response was substantially potentiated by the positive control drug, chlormethiazole, at a

30

concentration of 200 μ M (Figure 15a). In contrast, a concentration of 200 μ M of IVk produced a smaller, but still significant, potentiation of the control GABA response in the same oocyte (Figure 15a). A decreased efficacy with IVk suggests the possibility that it is a partial allosteric modulator of GABA_A receptor function, and may therefore have more selective behavioural effects than more efficacious compounds.

The effect of a drug that induces brief periods of sedation/hypnosis in animals is characterized by loss of the righting reflex (loss of the ability of the animal to right its posture when placed on its back). The "loss of the righting reflex" response is a commonly used test for sedative-hypnotic agents. IVk produced a dose-dependent and reversible loss of the righting reflex in mice when given by intraperitoneal injection at doses of 100 and 200 mg/kg (Figure 15b). Thus, the organic nitrate IVk exhibits the activity of a sedative/hypnotic, and represents a novel class of compounds that may have utility as new anti-anxiety, sedative and/or hypnotic agents.

Example 31: Synthesis of nitrate Vah

The title compound was synthesised by the reaction of nitrate IVs with 2-acetoxybenzoyl chloride in CHCl₃. Isolated yield 45%. (CDCl₃, 300 MHz): 8.21 (1H, dd, J 8.01, 1.62), 7.65 (1H, td, J 7.9, 1.63), 7.39 (1H, td, J 6.7, 1.1), 7.14 (1H, dd, J 8.07, 1.1), 5.29-5.38 (1H, m), 4.76 (1H, dd, J 12.94, 3.11), 4.55 (1H, dd, J 12.94, 6.37), 3.30 (1H, dd, J 14.06, 5.98), 3.13 (1H, dd, J 14.61, 6.35), 2.33 (3H, s). (CDCl₃, 75.48 MHz): 169.13, 164.58, 150.28, 136.05, 134.32, 126.44, 126.27, 124.19, 77.00, 69.79, 30.42, 20.79.

Example 32: Characterization of the cognition enhancing properties of a novel sedative-hypnotic nitrate ester

Adult male Long-Evans rats (250-300 g) were used to test spatial learning in the Morris water maze. A circular pool (1.83 m in diameter and 38 cm deep) was filled with water (21°C) made opaque by the addition of 500 ml of non-toxic white paint. A circular escape platform (25 cm high, 20 cm diameter) located about 2 cm below the water surface served as an escape from the water. The platform was placed in the center of one of the four pool quadrants away from the wall. Rats were given a total of 12 trials, grouped in 3 trial blocks (i.e., 4 trials/block; approximately 5 minutes between blocks). A trial started

with the rat being released into the pool facing the pool wall. For each trial block, rats were released from the four cardinal compass points by pseudo-random assignment. A trial lasted until the rat mounted the hidden platform, after which it was allowed to stay on the platform for 15 seconds. If the rat did not locate the platform after 60 seconds of swimming, it was manually guided to the platform and left there for 15 seconds before commencement of the next trial. The time to locate the hidden platform was measured by an experimenter standing in a fixed position relative to the pool. Different groups of rats received either scopolamine (0.5 mg/kg, i.p) + dimethylsulfoxide (vehicle) (1 ml/kg, s.c), or scopolamine + IVk (10 mg/kg, s.c.). Scopolamine was always administered 25 minutes before testing, and drug or vehicle injections were given 5 minutes later (20 minutes before testing). All drug administrations were given in a volume of 1 ml/kg body weight. Rats pretreated with scopolamine failed to learn the location of the hidden escape platform even with repeated trials (Table 3). At a dose of 10 mg/kg, IVk induced a reversal of scopolamine-impaired task acquisition in the Morris water maze (Table 3).

Table 3: Effect of IVk on scopolamine-impaired learning in the Morris water maze.

Trial Block	Vehicle Control	IVk (10 mg/kg)
1	56.64 \pm 2.10 (7)	54.21 \pm 2.45 (7)
2	50.43 \pm 3.43 (7)	38.86 \pm 3.41 (7), p < 0.05
3	57.96 \pm 1.14 (7)	37.64 \pm 3.09 (7), p < 0.01

Data represent the time to reach the hidden platform (mean \pm s.e.m. in seconds) for vehicle control and IVk-treated animals (number of animals shown in parentheses). Two-way analysis of variance revealed significant effects of drug treatment and trial block, suggesting that IVk produced a time-dependent reversal of the cognitive impairment caused by scopolamine.

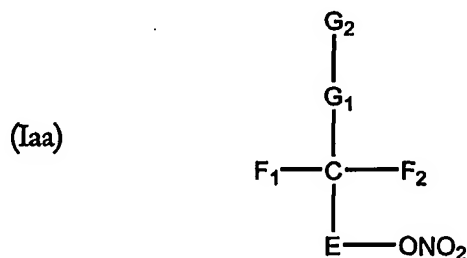
REFERENCES

- Aley *et al.*, *J. Neurosci.* (1998) 18, 7008.
- T.Azuhata, Y.Okamoto, *Synthesis* (1983) 916-917.
- Artz J.D., Thatcher, G.R.J., *Chem. Res. Toxicol.* (1998) 11, 1393.
- 5 • Bak *et al.*, *Life Sciences* (1998) 62, 367-373.
- Barger, SW, RR Fiscus, P Ruth, F Hofmann, MP Mattson, "Role of cyclic GMP in the regulation of neuronal calcium and survival by secreted forms of β -amyloid precursor protein", *J. Neurochem.* (1995) 64, 2087-2096.
- Bennett *et al.*, *Can. J. Physiol. Pharmacol.* (1992) 70, 1297.
- 10 • Berge *et al.*, "Pharmaceutical Salts", *J. Pharm. Sci.* (1977) 66, 1-19.
- Bloeman, P.G. *et al.*, *FEBS Lett.* (1995) 357, 140.
- Bourgeois, *Recl. Trav. Chim. Pays-Bas* (1899) 18, 445-450.
- Briscoe *et al.*, *Am. J Physiol.* (1995) 1233, 134.
- Cunha, F.Q. *et al.*, *Br. J Pharmacol.* (1999) 127, 671.
- 15 • del Soldato, P. *et al.*, *Trends Pharmacol. Sci.* (1999) 20, 319.
- Ferreira *et al.*, *Agents Actions Suppl.* (1991) 32, 101.
- Ferreira, S.H., *Drugs* (1993) 46, 1.
- Ferreira, S.H., *Ann. Ist Super Sanita* (1993) 29, 367.
- Fidecka, S. *et al.*, *Pol. J. Pharmacol.* (1997) 49, 395.
- 20 • R.L. Frank, P. V. Smith, *J. Am. Chem. Soc.* (1946) 68, 2103-2104.
- Granados-Soto *et al.*, *Eur. J. Pharmacol.* (1997) 340, 177.
- Inoue, T. *et al.*, *J. Neurol. Sci.* (1997) 153, 1.
- Lauretti, G.R. *et al.*, *Anesthesiology* (1999) 90, 1528.
- Louw, R., H.P.W. Vermeeren, J.J.A. Van Asten, W.J. Ultee, *J.Chem.Soc., Chem.Comm.*
- 25 (1976) 496-497.
- McDonald and Bennett, *Can. J. Physiol. Pharmacol* (1990) 68, 1552.
- McGuire *et al.*, *J. Pharmacol. Exp. Ther.* (1994) 271, 708.
- Malmberg and Yaksh, *Anesthesiology* (1993) 79, 270-281.
- Morgan *et al.*, "Approaches to the discovery of non-peptide ligands for peptide
- 30 receptors and peptidases", In *Ann. Rep. Med. Chem.* (Virick F.J., et al.) pp. 243-253, Academic Press, San Diego, CA (1989).

- J.-P. Morizur, *Bull. Soc. Chim. Fr.* (1964) 1338-1342.
- Ouellette, R.J., R. J. Bertsch, *J. Org. Chem.* (1976) 41, 2782-2783.
- Owais, M. *et al.*, *Antimicrob. Agents Chemother.* (1995) 39, 180.
- Ranade, V.V., *J. Clin. Pharmacol.* (1989) 29, 685.
- 5 • Reynolds and Maitra, *European Journal of Pharmacology* (1996) 314, 151-156.
- Salter, M. *et al.*, *Neuroscience* (1996) 73, 649.
- K.B.Sharpless, W.Amberg, Y.L.Bennani, G.A.Crispino, J.Hartung, K.-S.Jeong, H.-L.Kwong, K.Morikawa, Z.-M.Wang, D.Xu, X.-L.Zhang, *J.Org.Chem.* (1992) 57, 2768-2771.
- 10 • Shibuta, S., *J. Neurol. Sci.* (1996) 141, 1.
- Strejan *et al.*, *J Neuroimmunol.* (1984) 7, 27.
- Stewart *et al.*, *Can. J. Physiol Pharmacol.* (1989) 67, 403.
- J. R. Stone and M. A. Marletta, *Biochemistry* (1996) 35, 1093.
- Sydserff *et al.*, *Br. J. Pharmacol.* (1995) 114, 1631-1635.
- 15 • Tjolsen *et al.*, *Pain* (1992) 51, 5-17.
- Umezawa *et al.*, *Biochem. Biophys. Res. Commun.* (1988) 153, 1038.
- Wu, J, Y Wang, MJ Rowan, R Anwyl, "Evidence for involvement of the cGMP-protein kinase G signaling system in the induction of long-term depression, but not long-term potentiation, in the dentate gyrus *in vitro*", *J. Neurosci.* (1998) 18, 3589-3596.
- 20 • Xu J.Y. *et al.*, *Pain* (1995) 63, 377.
- Yaksh, *TIPS* (1999) 20, 329-337.
- Yang, K., J.D. Artz, J. Lock, C. Sanchez, B.M. Bennett, A.B. Fraser, G.R. Thatcher, *J.Chem.Soc., Perkin Trans.* (1996) 1, 1073-1075.

What is claimed is:

1. A method for treating pain, treating inflammation, or providing analgesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Iaa):



in which E, F₁, F₂, G₁, and G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

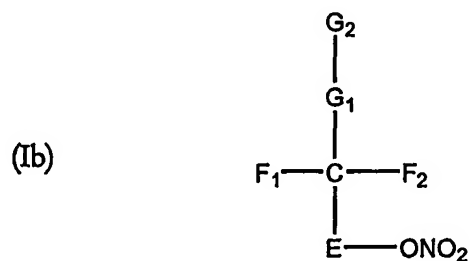
with the proviso that when E and G₁ are methylene groups and F₁ is H, G₂ is not a nitrate group, nor R^N-Z^N;

wherein R^N is any aryl or heteroaryl group and Z^N is (CO)_{mm}-X^N_{nn}-Y^N_{oo};

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

2. A method for treating pain, treating inflammation, or providing analgesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ib):



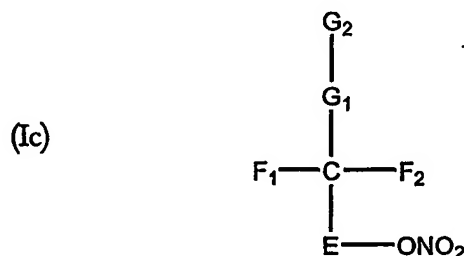
in which F_2 is an organic radical which may be joined in a cyclic ring system with G_2 , and which may contain inorganic counterions; E and G_1 are both methylene groups; F_1 is H; and G_2 is R^N-Z^N ;

wherein R^N is an organic radical possessing a heteroaryl group containing P or S atoms where said P or S are positioned β , γ , or δ to a nitrate group as identified in formula I; and Z^N is $W^N_{mm}-X^N_{nn}-Y^N_{oo}$;

wherein mm, nn and oo are 0 or 1; and W^N , X^N , Y^N are NH , NR^{NN} , CO , O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

3. A method for treating pain, treating inflammation, or providing analgesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ic):



in which E is $(R^1R^2C)_m$ and $G_2-G_1-CF_1F_2-$ is $R^{19}-(R^3R^4C)_p-(R^{17}R^{18}C)_n-$;

wherein: m, n, p are integers from 0 to 10;

$R^{3,17}$ are each independently hydrogen, a nitrate group, or A;

and

$R^{1,4}$ are each independently hydrogen, or A;

where A is selected from a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S, NR^6 and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or

substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between R^1 and R^3 and/or between R^{17} and R^4 , which optionally may contain O, S, NR^6 and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain) containing carbonyl linkages (e.g., $C=O$, $C=S$, $C=NOH$), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a substituted or unsubstituted heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties); arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy;

wherein X is F, Br, Cl, NO_2 , CH_2 , CF_2 , O, NH, NMe, CN, NHOH, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_2HM , PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)$, $C(O)R^{12}$, $C(O)(OR^{13})$, PO_2H , PO_2M , $P(O)(OR^{14})$, $P(O)(R^{13})$, SO, SO_2 , $C(O)(SR^{13})$, SR^5 , SSR^7 or SSR^5 ;

Y is F, Br, Cl, CH_3 , CF_2H , CF_3 , OH, NH_2 , NHR^6 , NR^6R^7 , CN, NHOH, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_2HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)R^{12}$, $C(O)(OR^{13})$, $C(O)(SR^{13})$, SR^5 , SSR^7 or SSR^5 , or does not exist;

R^2 , R^5 , R^{18} , R^{19} are optionally hydrogen, A, or X-Y;

R^6 , R^7 , R^8 , R^9 , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO_2 substituents; or $C_1 - C_6$ connections to $R^1 - R^4$ in cyclic derivatives which may contain 1-4 ONO_2 substituents; or are each independently hydrogen, a nitrate group, or A;

M is H, Na^+ , K^+ , NH_4^+ , $N^+H_kR^{11}_{(4-k)}$ where k is 0-3; or other pharmaceutically acceptable counterion;

and with the proviso that when $m = n = p = 1$ and R^{19} , R^2 , R^{18} , $R^1 = H$ and R^{17} , R^3 are nitrate groups, R^4 is not H.

4. The method of claim 3, in which E, F₁, F₂, G₁, and G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

5 wherein when m = 0, n=1, p=1, R¹⁷, R¹⁸, R³, R⁴ are H, C₁-C₁₂ alkyl, aryl, or alkylaryl groups, and R¹⁹ is not R^N-Z^N-;

wherein R^N-Z^N- is a substituted or unsubstituted aryl group or a substituted or unsubstituted heterocyclic group;

wherein R^N is any aryl or heteroaryl group and Z^N is (CO)_{mm}-X^N_{nn}-Y^N_{oo};

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

10 wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

5. The method of claim 1, wherein F₂ is a nitrate group; and E, F₁, G₁, G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

15 with the proviso that when E and G₁ are methylene groups and F₁ is H, G₂ is not a nitrate group, nor R^N-Z^N-;

wherein R^N is any aryl or heteroaryl group and Z^N is (CO)_{mm}-X^N_{nn}-Y^N_{oo};

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

20 6. The method of claim 2, wherein F₂ is a nitrate group; E and G₁ are methylene groups; F₁ is H; and G₂ is R^N-Z^N-;

wherein R^N is an organic radical possessing an heteroaryl group containing P or S atoms where said P or S are positioned β, γ, or δ to a nitrate group as identified in formula I; and Z^N is W^N_{mm}-X^N_{nn}-Y^N_{oo};

25 wherein mm, nn, oo are 0 or 1 and W^N, X^N, Y^N are NH, NR^{NN}, CO, O or CH₂;

wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

7. The method of claim 3, wherein R¹⁹ is X-Y.

30 8. The method of claim 7, wherein:

R^1 and R^3 are the same or different and selected from H and C_1 - C_4 alkyl chains, which chains may include one O linking R^1 and R^3 to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;

R^2 and R^4 are the same or different and selected from H, a nitrate group, C_1 - C_4 alkyl chains optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R^5$);

R^7 , R^{11} are the same or different C_1 - C_8 alkyl or acyl;

R^5 , R^6 , R^8 , R^9 , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} are the same or different and are alkyl groups containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents; or C_1 or C_2 connections to R^1 - R^3 in cyclic derivatives; and

M is H, Na^+ , K^+ , NH_4^+ or $N^+H_kR^{11}_{(4-k)}$, where k is 0-3.

9. The method of claim 7, wherein $m = 1$, $n = 1$, $p = 1$.

10. The method of claim 9, wherein:

X is CH_2 , O, NH, NMe, CN, NHOH, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)$, $C(O)R^{12}$, $C(O)(OR^{13})$, PO_2M , $P(O)(OR^{14})$, $P(O)(R^{13})$, SO, SO_2 , $C(O)(SR^{13})$, or SSR^5 ; and

Y is CN, $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , SCN, $SCN_2H_2(R^{15})_2$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SR^7 , SO_2M , PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)R^{12}$, $C(O)(SR^{13})$, SR^5 , or SSR^5 , or does not exist.

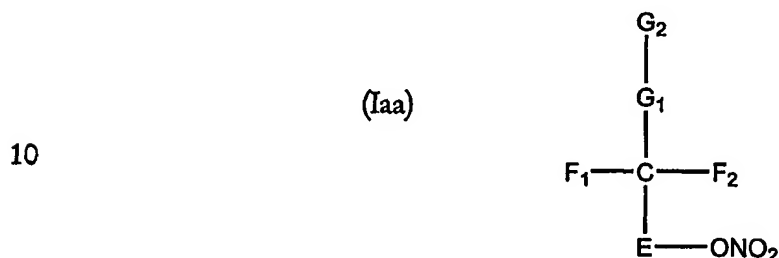
11. The method of claim 9, wherein:

R^5 , R^6 , R^8 , R^9 , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} are the same or different and are alkyls containing 1-12 carbon atoms; or C_1 or C_2 connections to R^1 or R^3 in cyclic derivatives;

X is CH_2 , O, NH, NMe, S, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, PO_3HM or $P(O)(OM)R^{15}$; and

Y is SO_2M , SO_3M , PO_3HM , PO_3M_2 , $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$, $\text{P}(\text{O})(\text{OR}^{16})(\text{OM})$, SR^5 , SR^7 or SSR^5 , or does not exist.

12. A method for providing sedation, mitigating anxiety, or providing anaesthesia in a subject in need thereof, comprising administering to a subject an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Iaa):



in which E, F₁, F₂, G₁, G₂ are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions, but which do not contain an organic nitrate group;

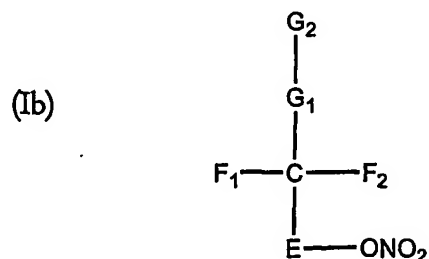
with the proviso that when E and G₁ are methylene groups and F₁ is H, G₂ is not a nitrate group, nor R^N-Z^N;

wherein R^N is any aryl or heteroaryl group and Z^N is $(\text{CO})_{\text{mm}}-\text{X}^{\text{N}}-\text{Y}^{\text{N}}_{\text{oo}}$;

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN}, O or CH₂;

wherein R^{NN} is a short chain alkyl group (C₁ - C₁₂).

13. A method for providing sedation, mitigating anxiety, or providing anaesthesia in a subject in need thereof, comprising administering to a subject an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ib):



in which F_2 is an organic radical which may be joined in a cyclic ring system with G_2 ,

and which may contain inorganic counterions, but is not a nitrate group; E and G_1 are methylene groups; F_1 is H; and G_2 is R^N-Z^N ;

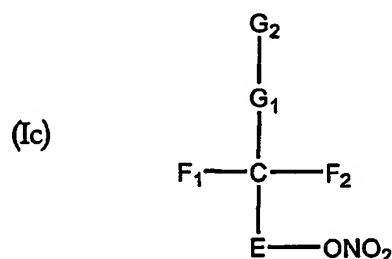
5 wherein R^N is an organic radical possessing a heteroaryl group containing P or S atoms

where said P or S are positioned β , γ , or δ to a nitrate group as identified in formula I; and Z^N is $W^N-X^N-Y^N$;

wherein mm, nn, oo are 0 or 1 and W^N, X^N, Y^N are NH, NR^{NN}, CO, O or CH_2 ;

10 wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

14. A method for providing sedation, mitigating anxiety, or providing anaesthesia in a subject in need thereof, comprising administering to a subject an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ic):



in which E is $(R^1R^2C)_m$ and $G_2-G_1-CF_1F_2-$ is $R^{19}-(R^3R^4C)_p-(R^{17}R^{18}C)_n-$;

wherein: m, n, p are integers from 0 to 10;

$R^{3,17}$ are each independently hydrogen, a nitrate group, or A;

25 and

$R^{1,4}$ are each independently hydrogen, or A;

where A is selected from a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), which optionally may contain O, S, NR^6 and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring,

30

which optionally may contain O, S, NR⁶ and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between R¹ and R³ and/or between R¹⁷ and R⁴, which optionally may contain O, S, NR⁶ and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain) containing carbonyl linkages (e.g., C=O, C=S, C=NOH), which optionally may contain O, S, NR⁶ and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a substituted or unsubstituted heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy;

wherein X is F, Br, Cl, NO₂, CH₂, CF₂, O, NH, NMe, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₂HM, PO₃HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O), C(O)R¹², C(O)(OR¹³), PO₂H, PO₂M, P(O)(OR¹⁴), P(O)(R¹³), SO, SO₂, C(O)(SR¹³), SR⁵, SSR⁷ or SSR⁵;

Y is F, Br, Cl, CH₃, CF₂H, CF₃, OH, NH₂, NHR⁶, NR⁶R⁷, CN, NHOH, N₂H₃, N₂H₂R¹³, N₂HR¹³R¹⁴, N₃, S, SCN, SCN₂H₂(R¹⁵)₂, SCN₂H₃(R¹⁵), SC(O)N(R¹⁵)₂, SC(O)NHR¹⁵, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₂HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), P(O)(OM)R¹⁵, CO₂M, CO₂H, CO₂R¹¹, C(O)R¹², C(O)(OR¹³), C(O)(SR¹³), SR⁵, SSR⁷ or SSR⁵, or does not exist;

R², R⁵, R¹⁸, R¹⁹ are optionally hydrogen, A, or X-Y;

R⁶, R⁷, R⁸, R⁹, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶ are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO₂ substituents; or C₁ - C₆ connections to R¹ - R⁴ in cyclic derivatives which may contain 1-4 ONO₂ substituents; or are each independently hydrogen, a nitrate group, or A;

M is H, Na⁺, K⁺, NH₄⁺, N⁺H_kR¹¹_(4-k) where k is 0-3; or other pharmaceutically acceptable counterion;

and with the proviso that when $m = n = p = 1$ and $R^{19}, R^2, R^{18}, R^1 = H$ and R^{17}, R^3 are nitrate groups, R^4 is not H.

15. The method of claim 14, in which E, F_1, F_2, G_1 , and G_2 are the same or different
5 organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

wherein when $m = 0, n = 1, p = 1, R^{17}, R^{18}, R^3, R^4$ are H, C_1 - C_{12} alkyl, aryl, or alkylaryl groups, and R^{19} is not R^N-Z^N ;

- 10 wherein R^N-Z^N is a substituted or unsubstituted aryl group or a substituted or unsubstituted heterocyclic group;

wherein R^N is any aryl or heteroaryl group and Z^N is $(CO)_{mm}-X_{nn}^N-Y_{oo}^N$;

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN} , O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

- 15 16. The method of claim 12, wherein F_2 is a nitrate group; and E, F_1, G_1, G_2 are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

with the proviso that when E and G_1 are methylene groups and F_1 is H, G_2 is not a nitrate group, nor R^N-Z^N ;

- 20 wherein R^N is any aryl or heteroaryl group and Z^N is $(CO)_{mm}-X_{nn}^N-Y_{oo}^N$;

wherein mm, nn, oo are 0 or 1 and X^N, Y^N are NH, NR^{NN} , O or CH_2 ;

wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

17. The method of claim 13, wherein F_2 is a nitrate group; E and G_1 are methylene
25 groups; F_1 is H; and G_2 is R^N-Z^N ;

wherein R^N is an organic radical possessing an heteroaryl group containing P or S atoms where said P or S are positioned β, γ , or δ to a nitrate group as identified in formula I; and Z^N is $W_{mm}^N-X_{nn}^N-Y_{oo}^N$;

wherein mm, nn, oo are 0 or 1 and W^N, X^N, Y^N are NH, NR^{NN} , CO, O or CH_2 ;

- 30 wherein R^{NN} is a short chain alkyl group ($C_1 - C_{12}$).

18. The method of claim 14, wherein R^{19} is X-Y.

19. The method of claim 18, wherein:

R^1 and R^3 are the same or different and selected from H and C_1 - C_4 alkyl chains, which chains may include one O linking R^1 and R^3 to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;

R^2 and R^4 are the same or different and selected from H, a nitrate group, C_1 - C_4 alkyl chains optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R^5$);

R^7 , R^{11} are the same or different C_1 - C_8 alkyl or acyl;

R^5 , R^6 , R^8 , R^9 , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} are the same or different and are alkyl groups containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents; or C_1 or C_2 connections to R^1 - R^3 in cyclic derivatives; and

M is H, Na^+ , K^+ , NH_4^+ or $N^+H_kR^{11}_{(4-k)}$, where k is 0-3.

20. The method of claim 18, wherein $m = 1$, $n = 1$, $p = 1$.

21. The method of claim 20, wherein:

X is CH_2 , O, NH, NMe, CN, NHOH, N_2H_3 , $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , S, SCN, $SCN_2H_2(R^{15})_2$, $SCN_2H_3(R^{15})$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SH, SR^7 , SO_2M , $S(O)R^8$, $S(O)_2R^9$, $S(O)OR^8$, $S(O)_2OR^9$, PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)$, $C(O)R^{12}$, $C(O)(OR^{13})$, PO_2M , $P(O)(OR^{14})$, $P(O)(R^{13})$, SO, SO_2 , $C(O)(SR^{13})$, or SSR^5 ; and

Y is CN, $N_2H_2R^{13}$, $N_2HR^{13}R^{14}$, N_3 , SCN, $SCN_2H_2(R^{15})_2$, $SC(O)N(R^{15})_2$, $SC(O)NHR^{15}$, SO_3M , SR^7 , SO_2M , PO_3HM , PO_3M_2 , $P(O)(OR^{15})(OR^{16})$, $P(O)(OR^{16})(OM)$, $P(O)(R^{15})(OR^8)$, $P(O)(OM)R^{15}$, CO_2M , CO_2H , CO_2R^{11} , $C(O)R^{12}$, $C(O)(SR^{13})$, SR^5 , or SSR^5 , or does not exist.

22. The method of claim 20, wherein:

R^5 , R^6 , R^8 , R^9 , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} are the same or different and are alkyls containing 1-12 carbon atoms; or C_1 or C_2 connections to R^1 or R^3 in cyclic derivatives;

X is CH₂, O, NH, NMe, S, SO₃M, SH, SR⁷, SO₂M, S(O)R⁸, S(O)₂R⁹, S(O)OR⁸, S(O)₂OR⁹, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), P(O)(R¹⁵)(OR⁸), PO₃HM or P(O)(OM)R¹⁵; and

Y is SO₂M, SO₃M, PO₃HM, PO₃M₂, P(O)(OR¹⁵)(OR¹⁶), P(O)(OR¹⁶)(OM), SR⁵, SR⁷
 5 or SSR⁵, or does not exist.

23. The method of claim 3, with the proviso that when $m = n = p = 1$ and R¹⁹, R², R¹⁸, R¹ = H and R¹⁷, R³ are nitrate groups, R⁴ is not C₁ - C₃ alkyl.

10 24. The method of claim 14, with the proviso that when $m = n = p = 1$ and R¹⁹, R², R¹⁸, R¹ = H and R¹⁷, R³ are nitrate groups, R⁴ is not C₁ - C₃ alkyl.

25. The method of any one of claims 1, 2, 3, 4, 5, or 6, further comprising administering the therapeutic compound with a pharmaceutically acceptable vehicle.

15

26. The method of any one of claims 12, 13, 14, 15, 16, or 17, further comprising administering the therapeutic compound with a pharmaceutically acceptable vehicle.

27. The method of any one of claims 1, 2, 3, 4, 5, or 6, wherein the therapeutic
 20 compound modulates levels of the cyclic nucleotides cGMP and/or cAMP in said subject.

28. The method of any one of claims 12, 13, 14, 15, 16, or 17, wherein the therapeutic compound modulates levels of the cyclic nucleotides cGMP and/or cAMP in said subject.

25 29. The method of any one of claims 1, 2, 3, 4, 5, or 6, wherein the therapeutic compound modulates guanylyl cyclase activity in said subject.

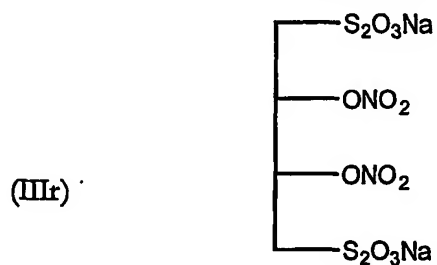
30. The method of any one of claims 12, 13, 14, 15, 16, or 17, wherein the therapeutic compound modulates guanylyl cyclase activity in said subject.

30

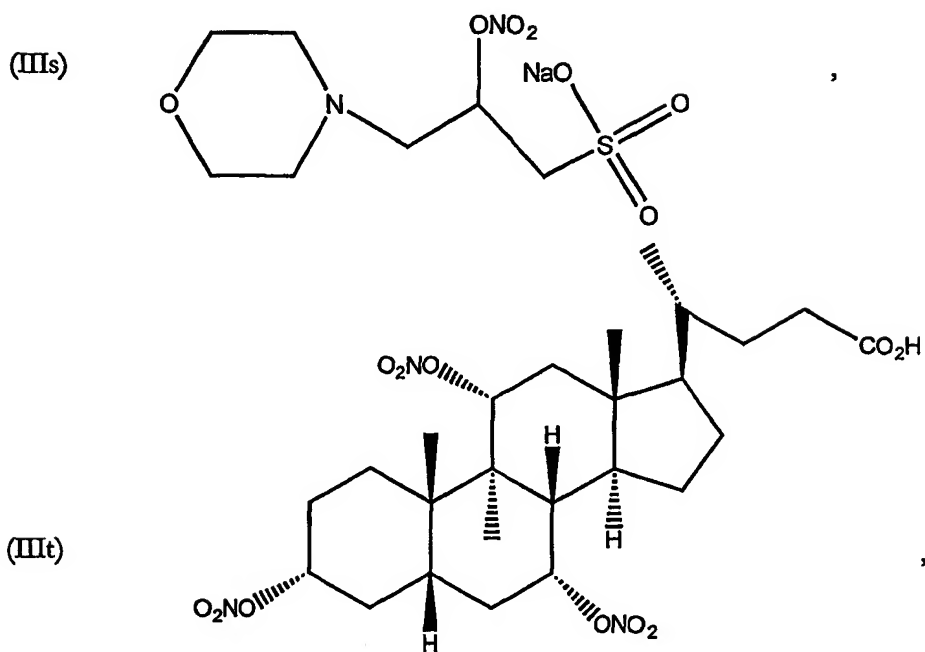
31. The method of any one of claims 1, 2, 4, 5, 6, 12, 13, 15, 16, and 17 wherein R^{NN} is a short chain alkyl group ($C_1 - C_8$).

32. A compound selected from the group consisting of

5



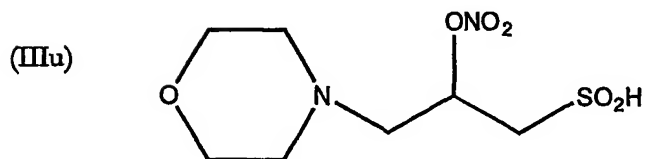
10



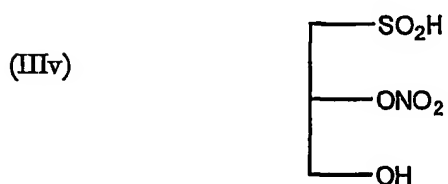
15

20

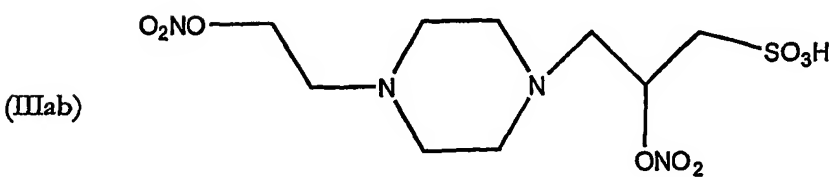
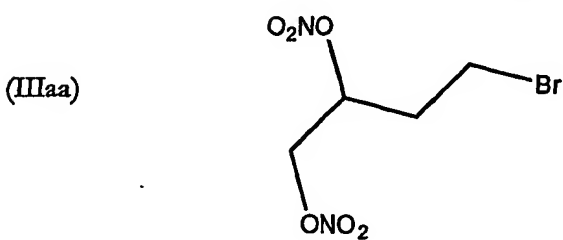
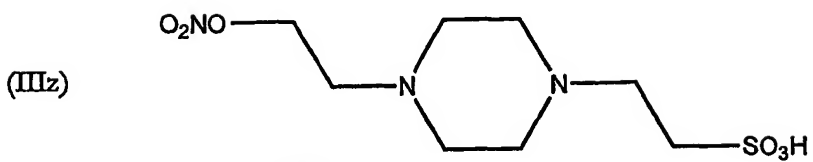
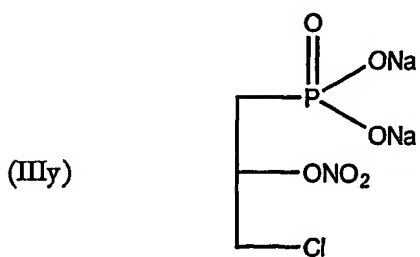
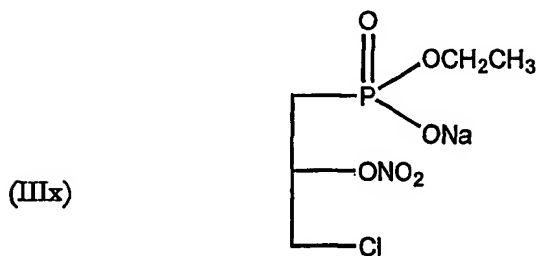
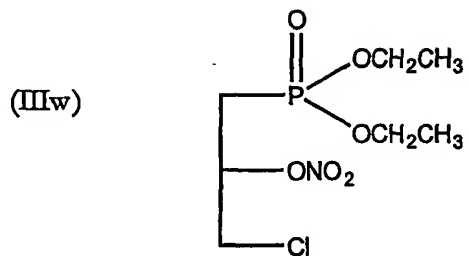
25



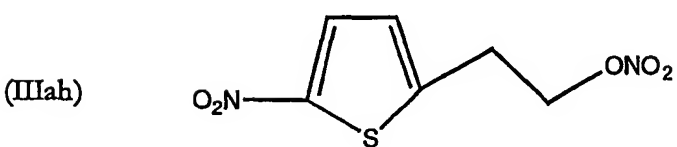
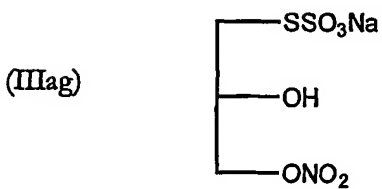
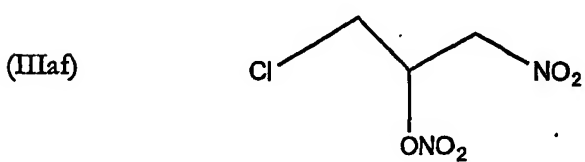
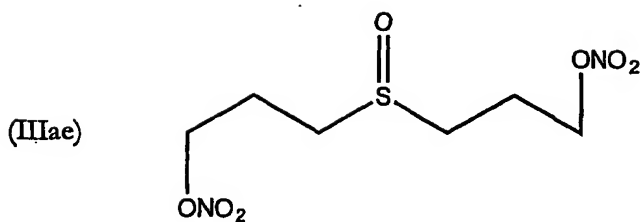
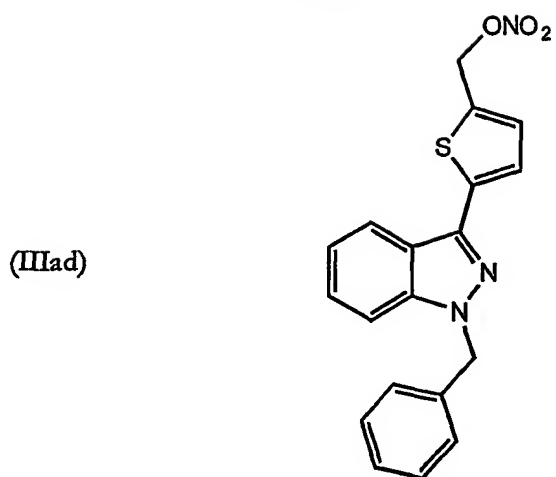
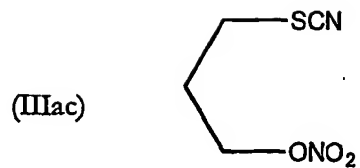
30



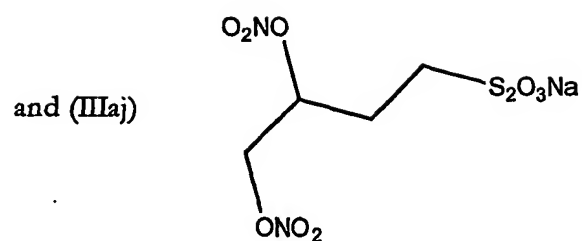
89



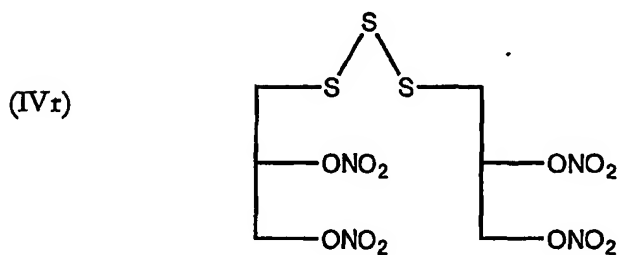
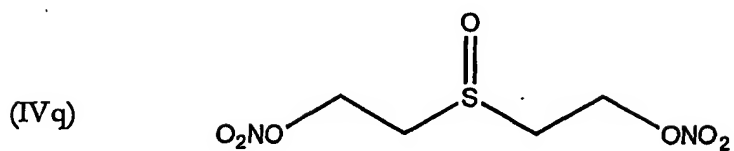
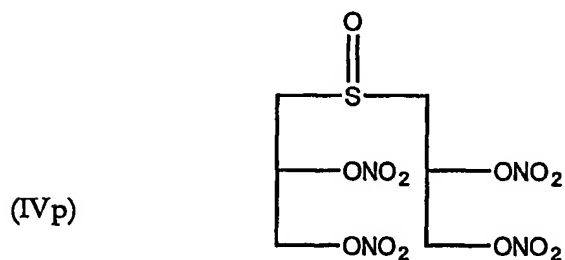
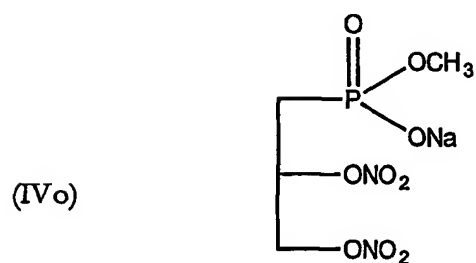
90



91

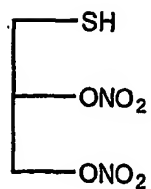


33. A compound selected from the group consisting of



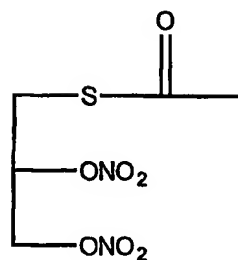
92

(IVs)



5

and (IVt)

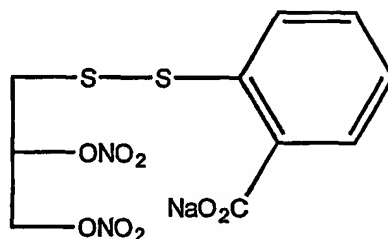


10

34. A compound selected from the group consisting of

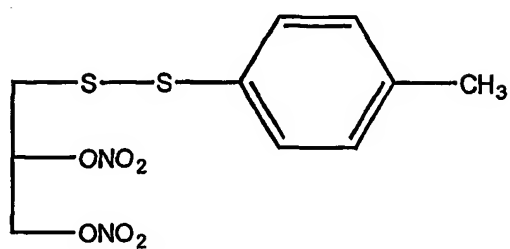
15

(Vd)



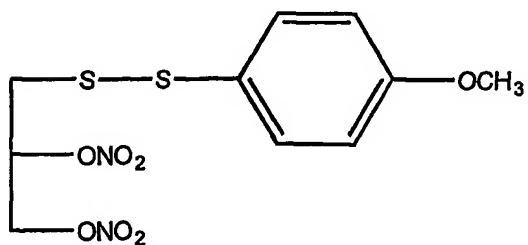
20

(Ve)



25

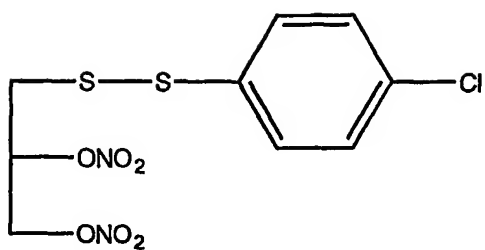
(Vf)



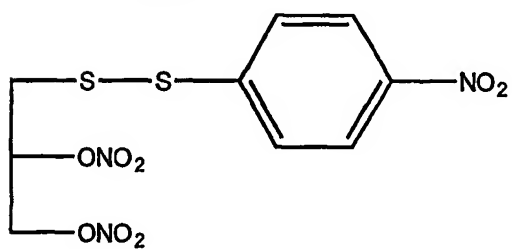
30

93

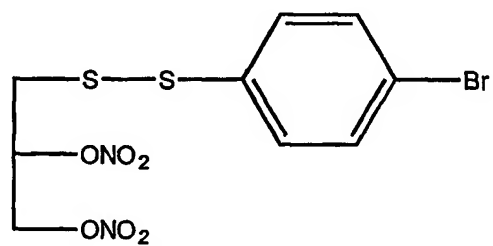
(Vg)



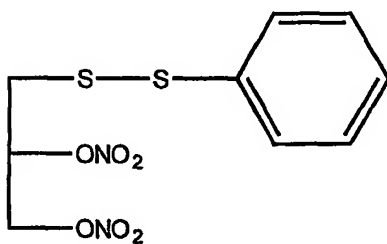
(Vh)



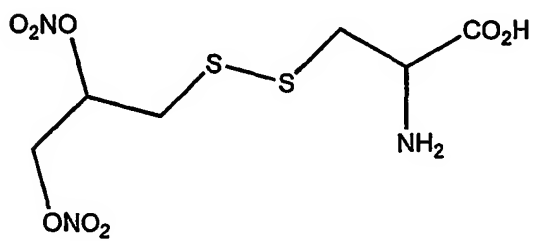
(Vi)



(Vj)



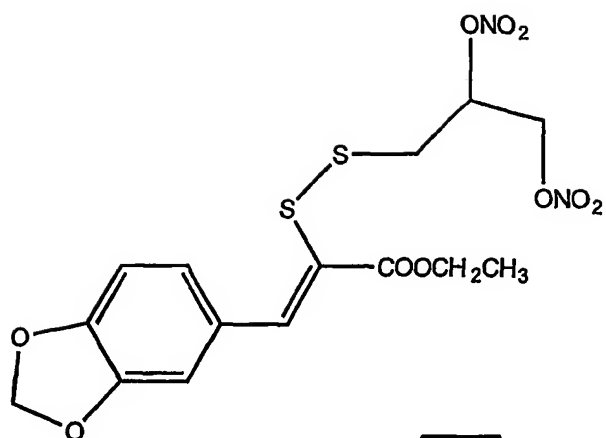
(Vk)



94

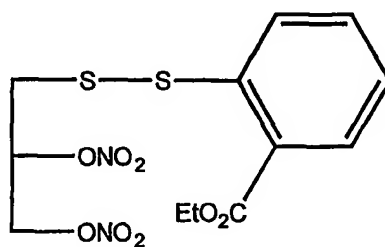
5

(VI)



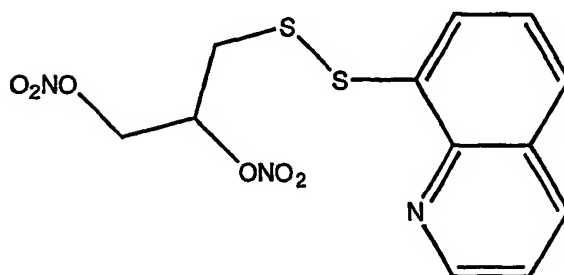
10

(Vm)



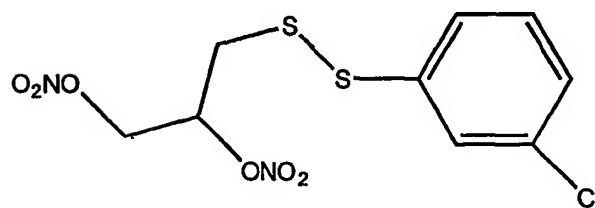
15

(Vn)



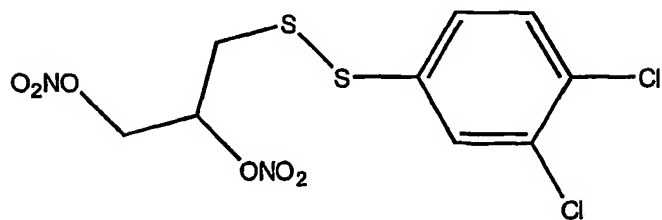
20

(Vo)



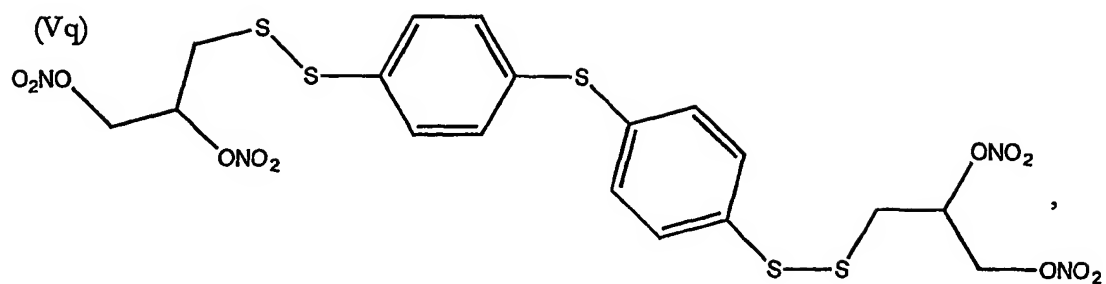
25

(Vp)



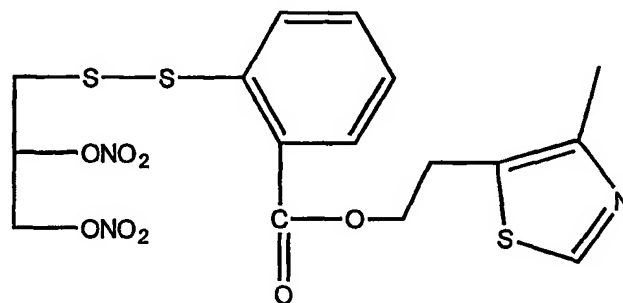
30

95



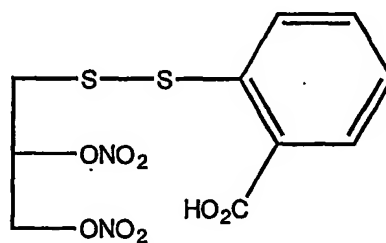
(Vr)

10



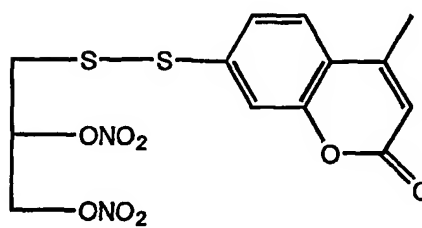
(Vs)

15



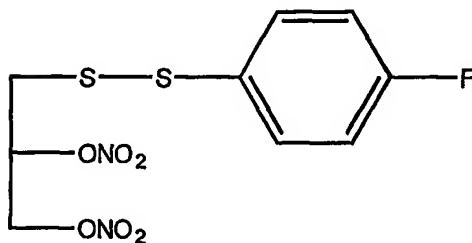
(Vt)

20



(Vu)

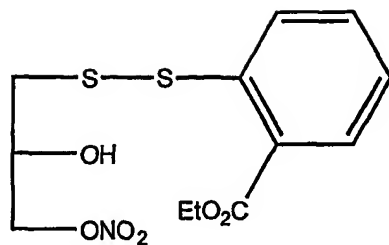
25



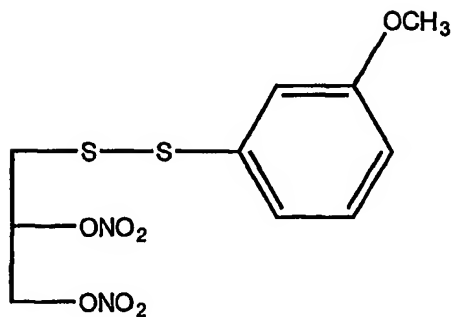
30

96

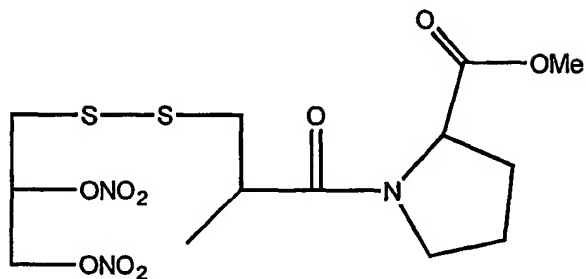
(Vv)



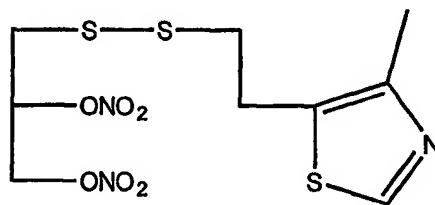
(Vw)



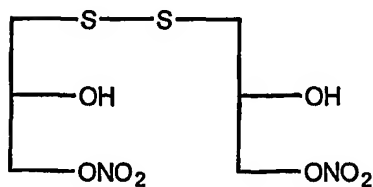
(Vx)



(Vy)

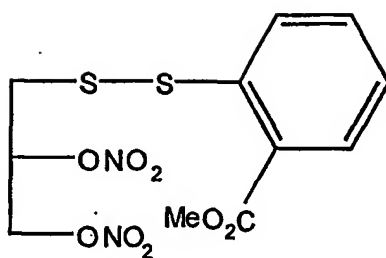


(Vz)

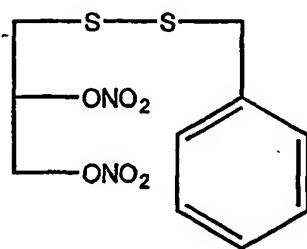


97

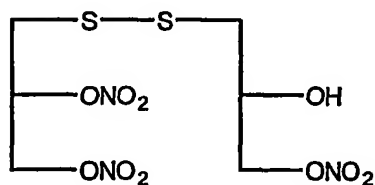
(Vaa)



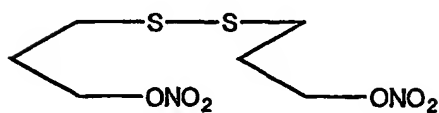
(Vab)



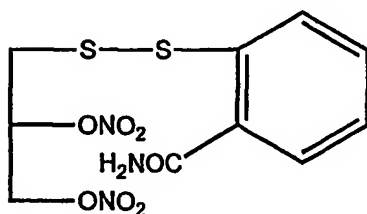
(Vac)



(Vad)

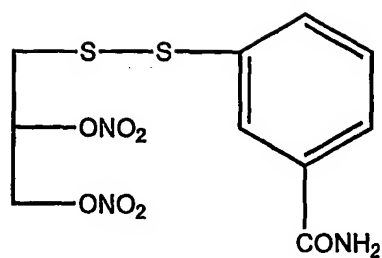


(Vae)



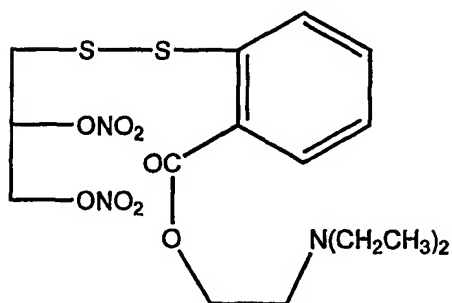
98

(Vaf)



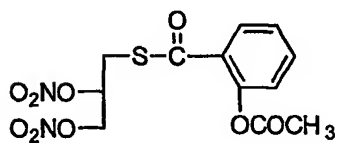
5

(Vag)



10

and (Vah)

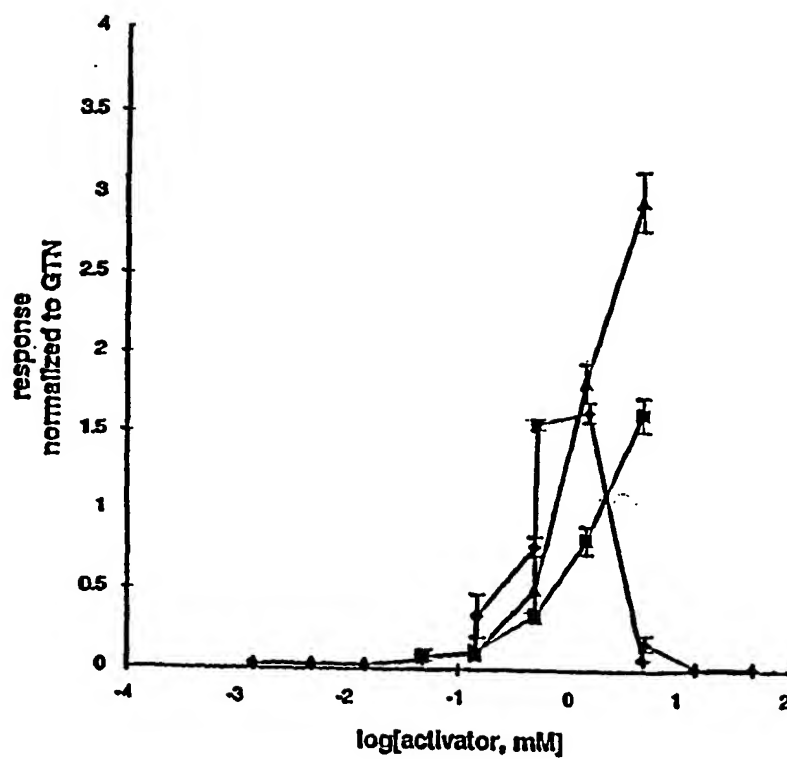


15

- 20 35. A pharmaceutical composition comprising a said compound of any one of claims 32, 33, and 34 and a pharmaceutically acceptable vehicle.

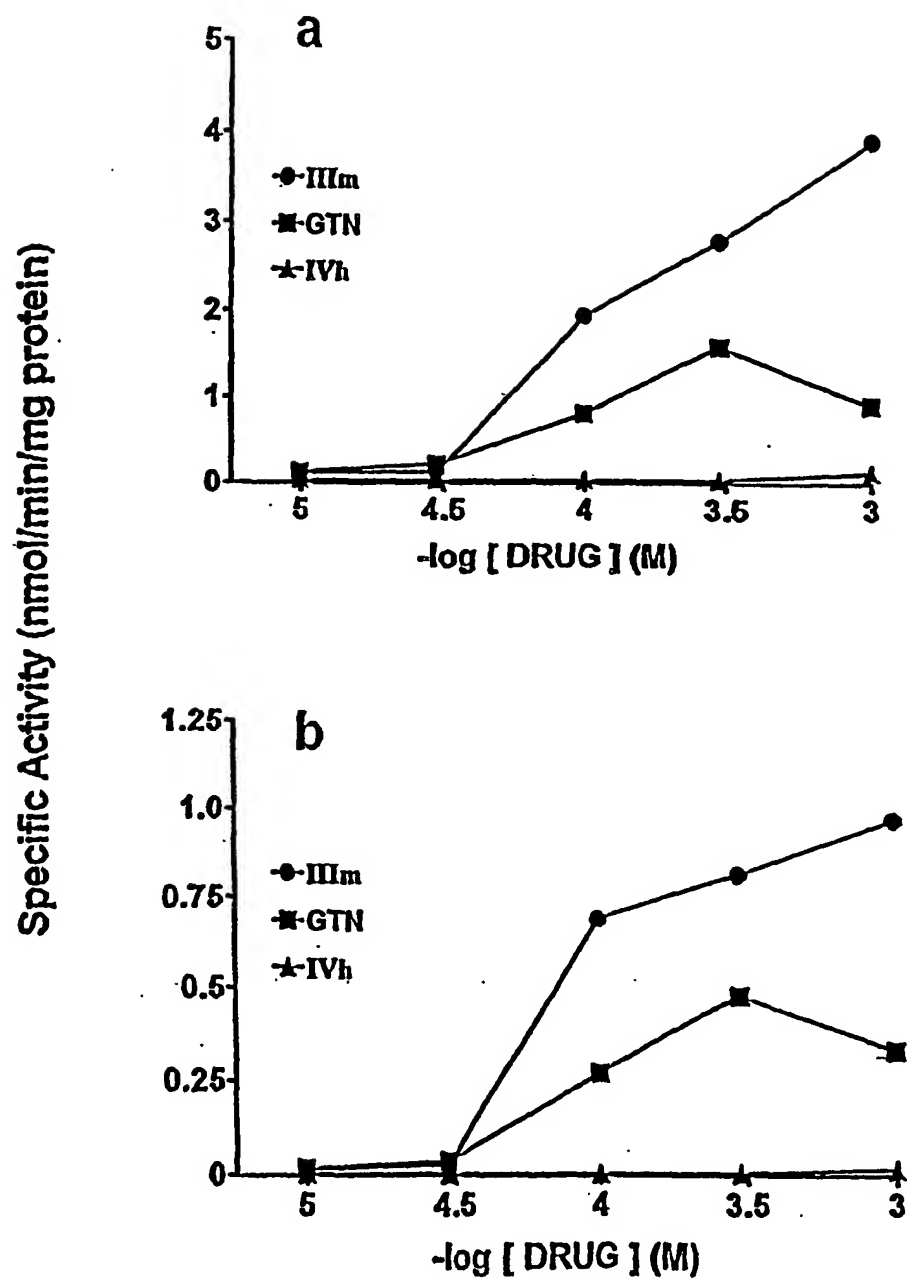
1/15

FIGURE 1



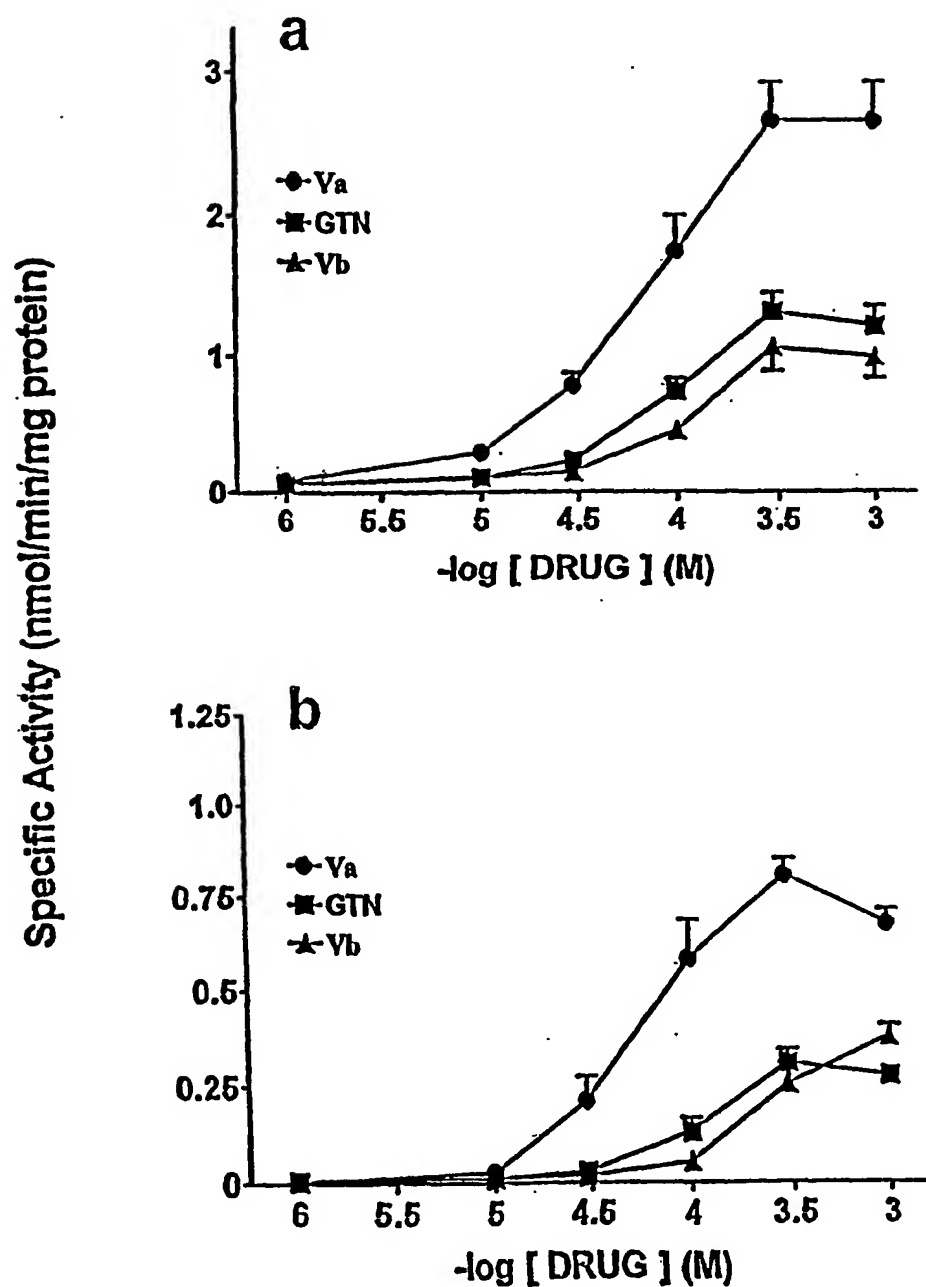
2/15

FIGURE 2



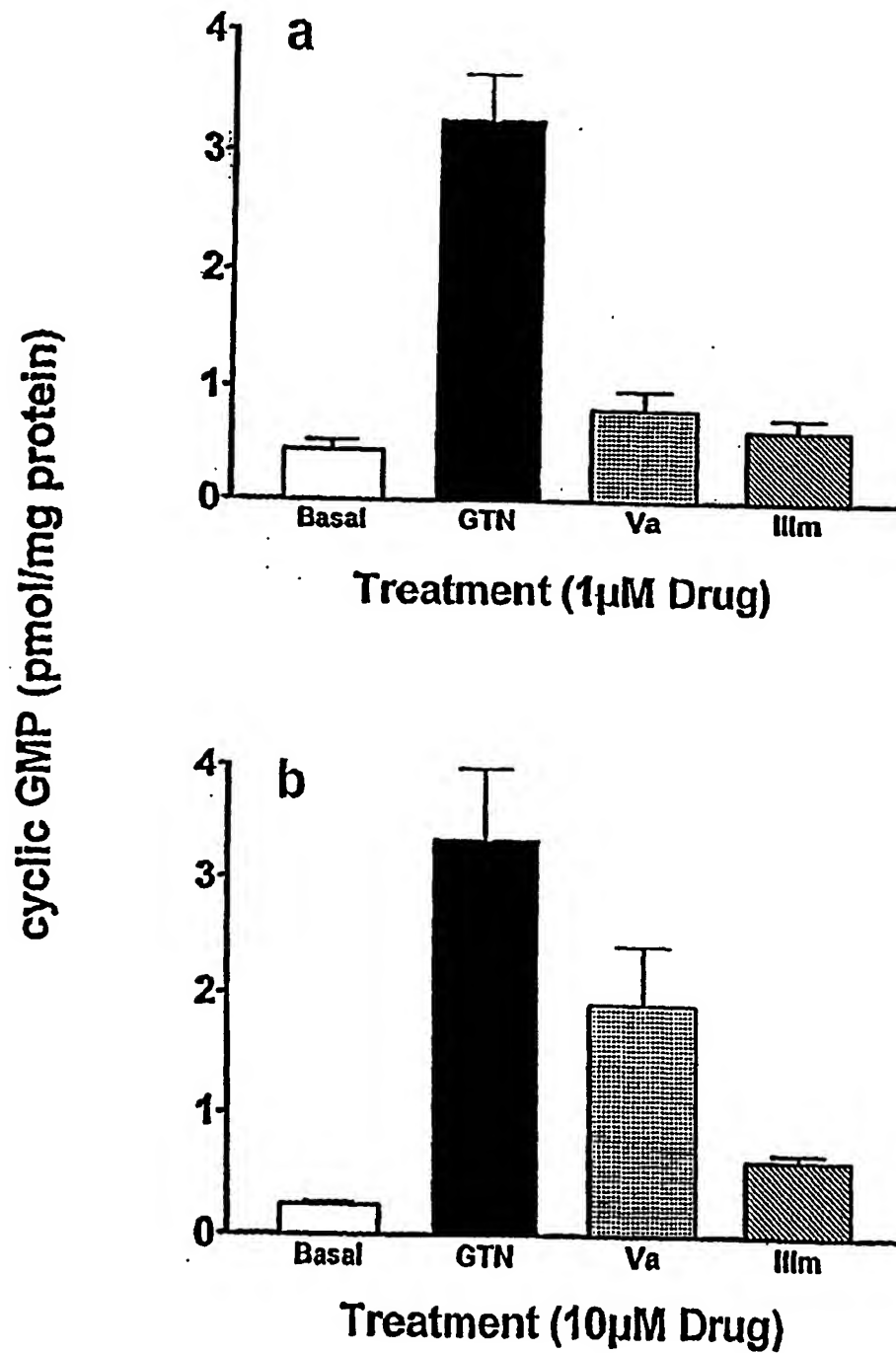
3/15

FIGURE 3



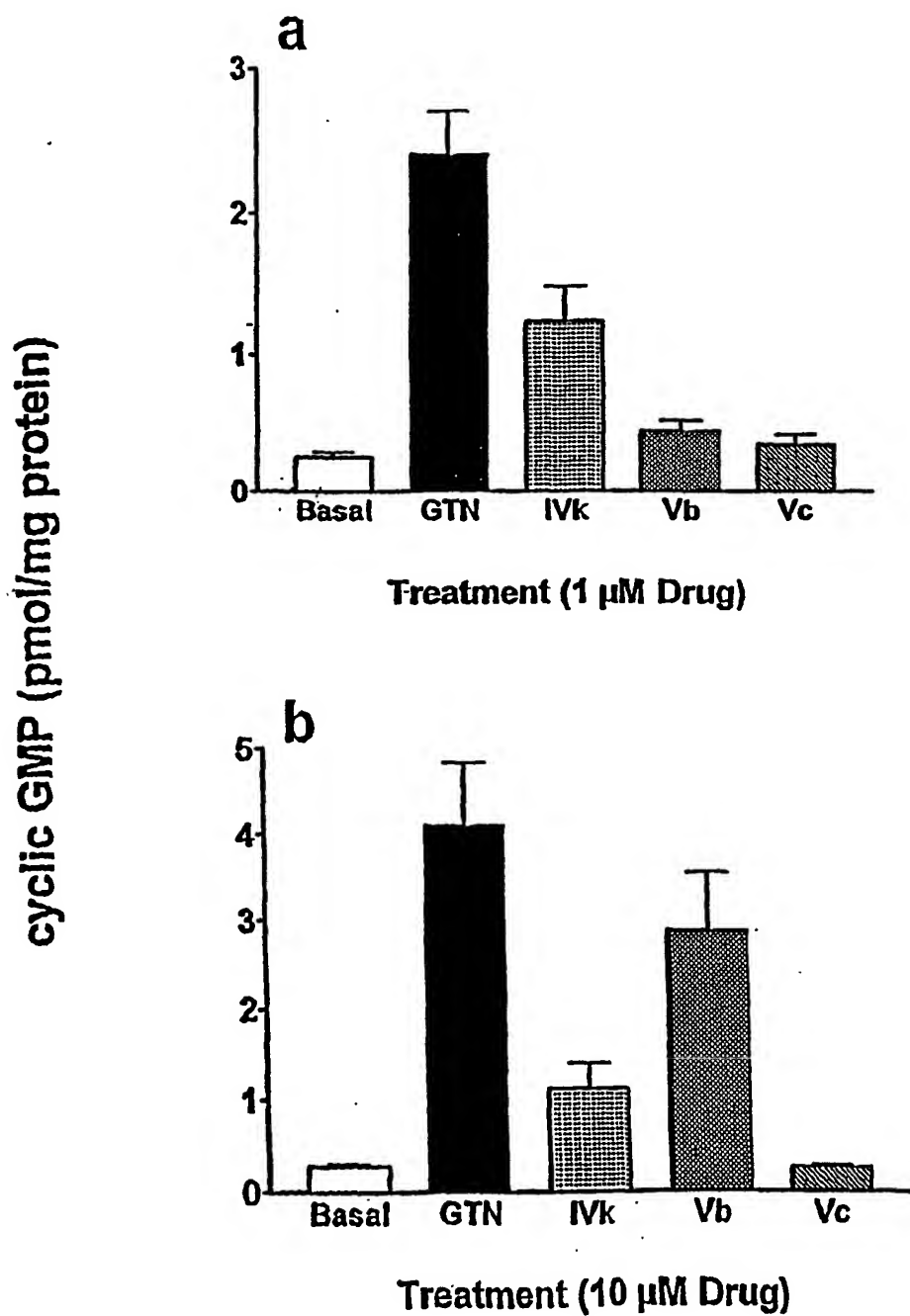
4/15

FIGURE 4



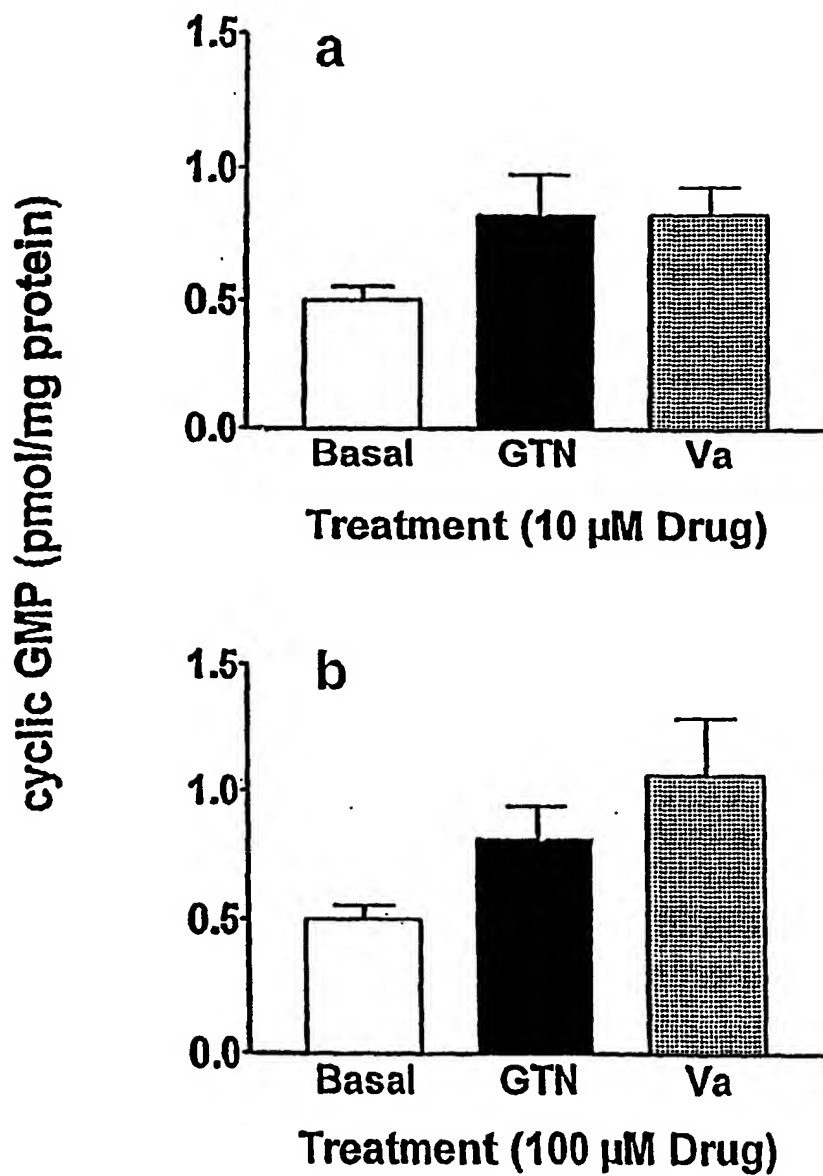
5/15

FIGURE 5



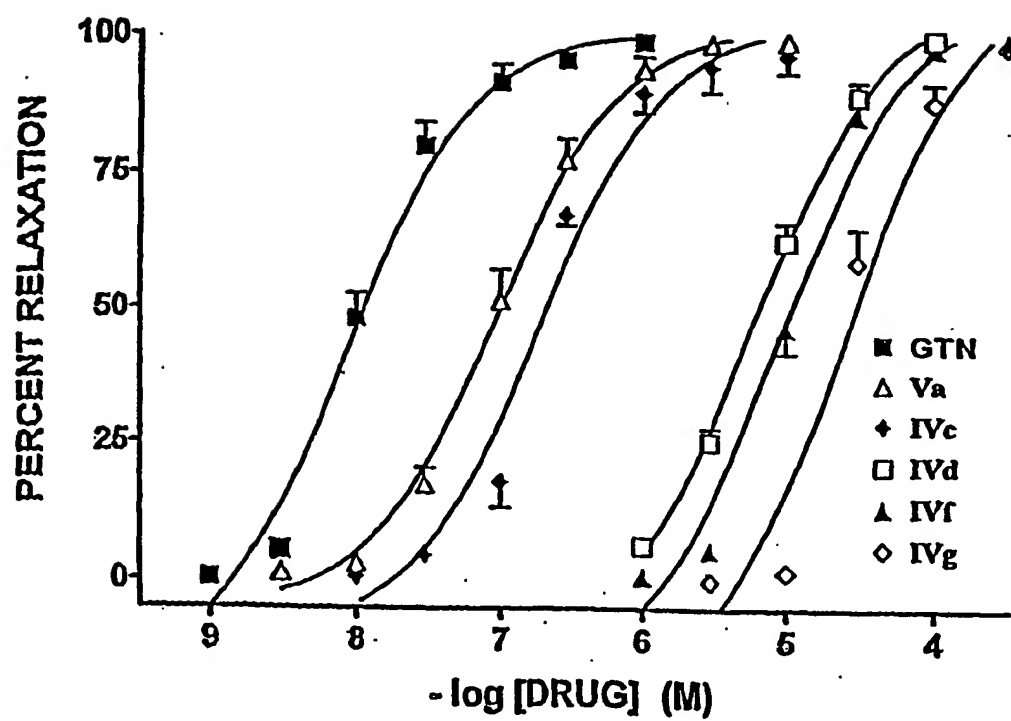
6/15

FIGURE 6



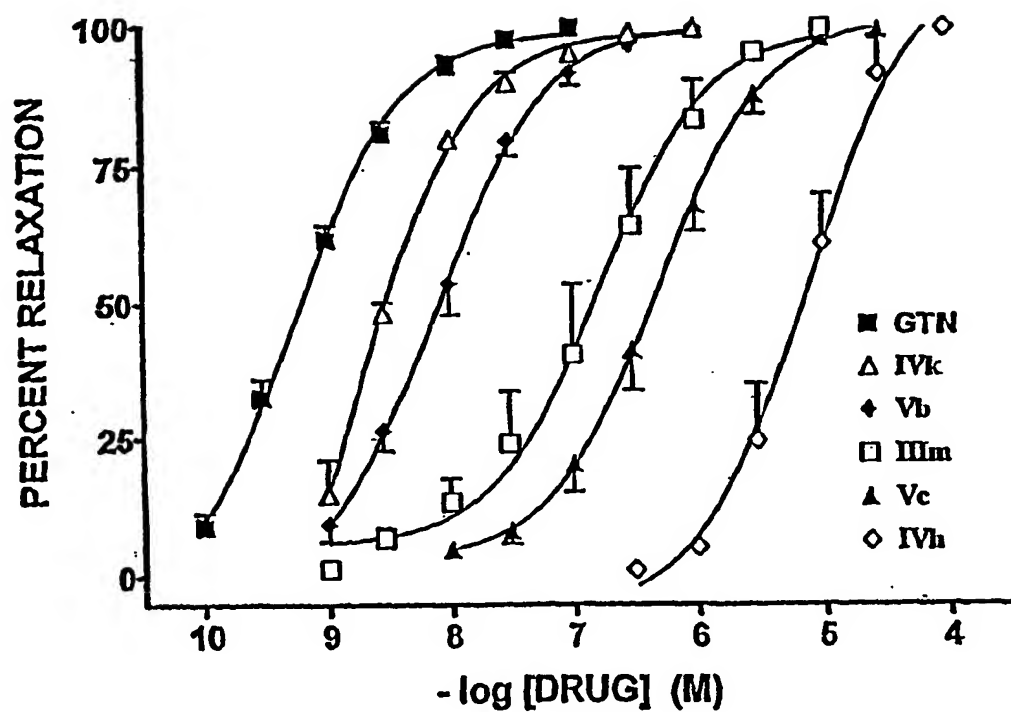
7/15

FIGURE 7



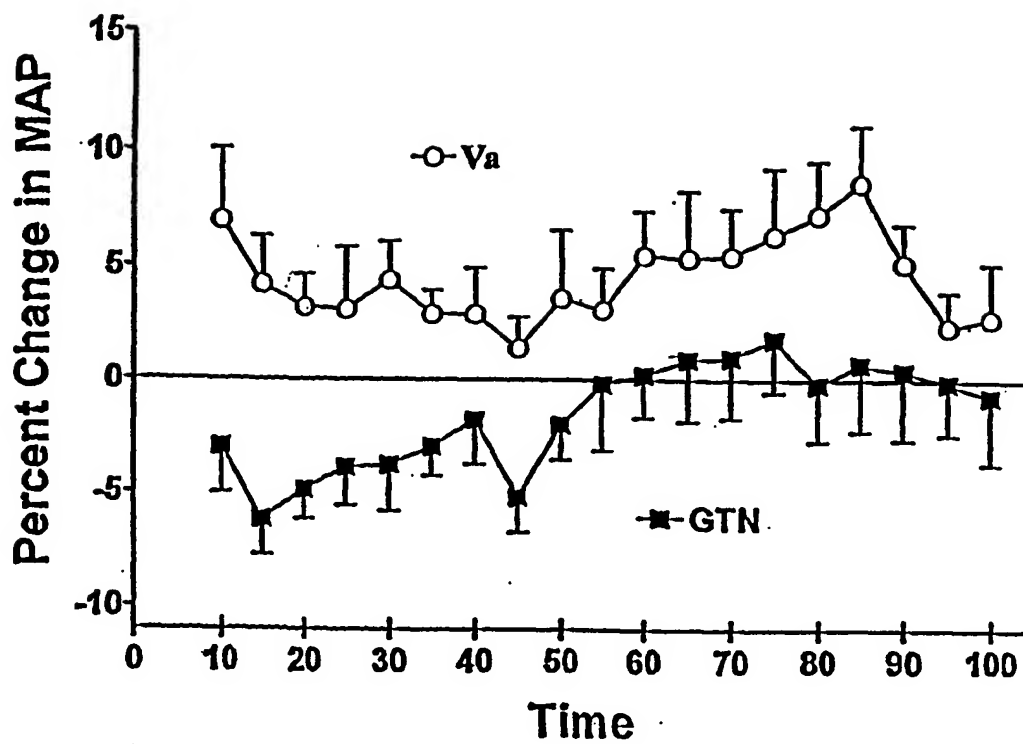
8/15

FIGURE 8



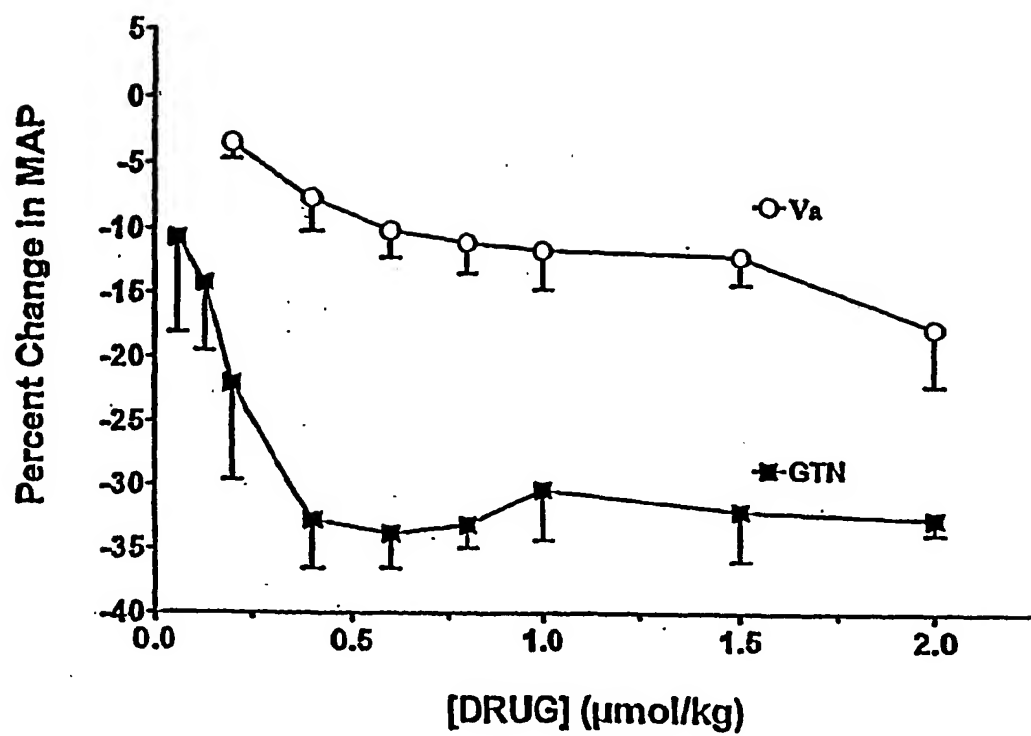
9/15

FIGURE 9



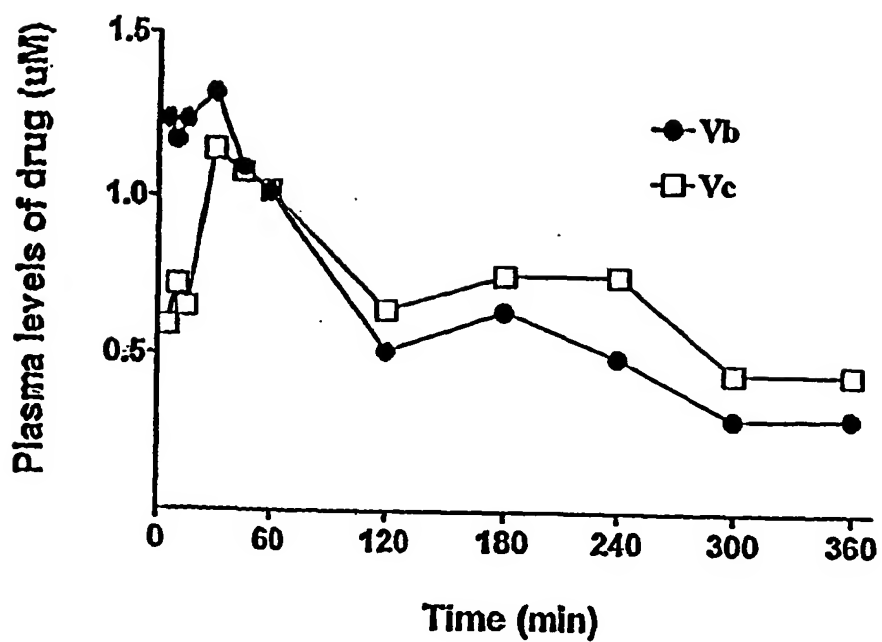
10/15

FIGURE 10



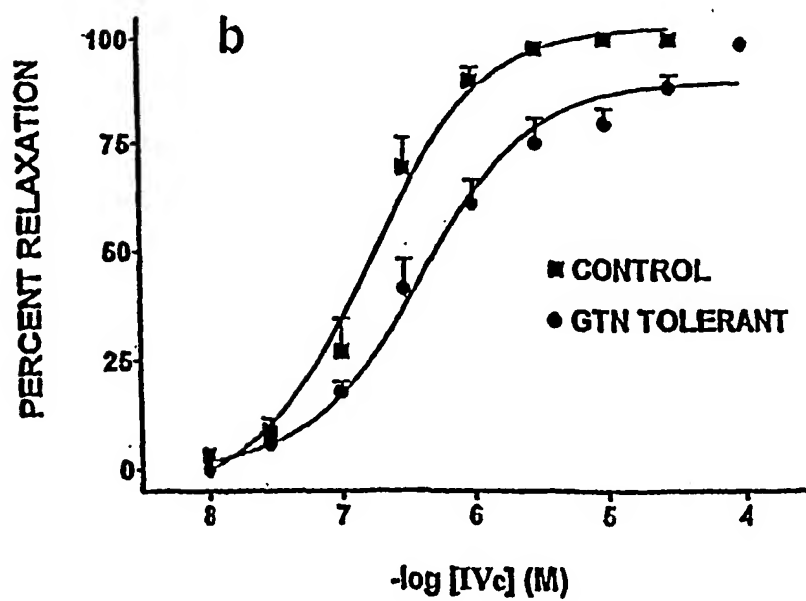
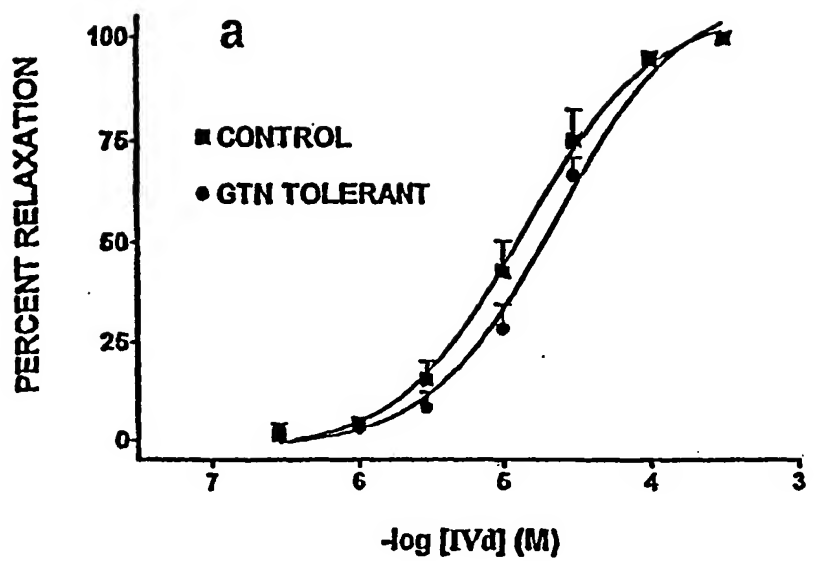
11/15

FIGURE 11



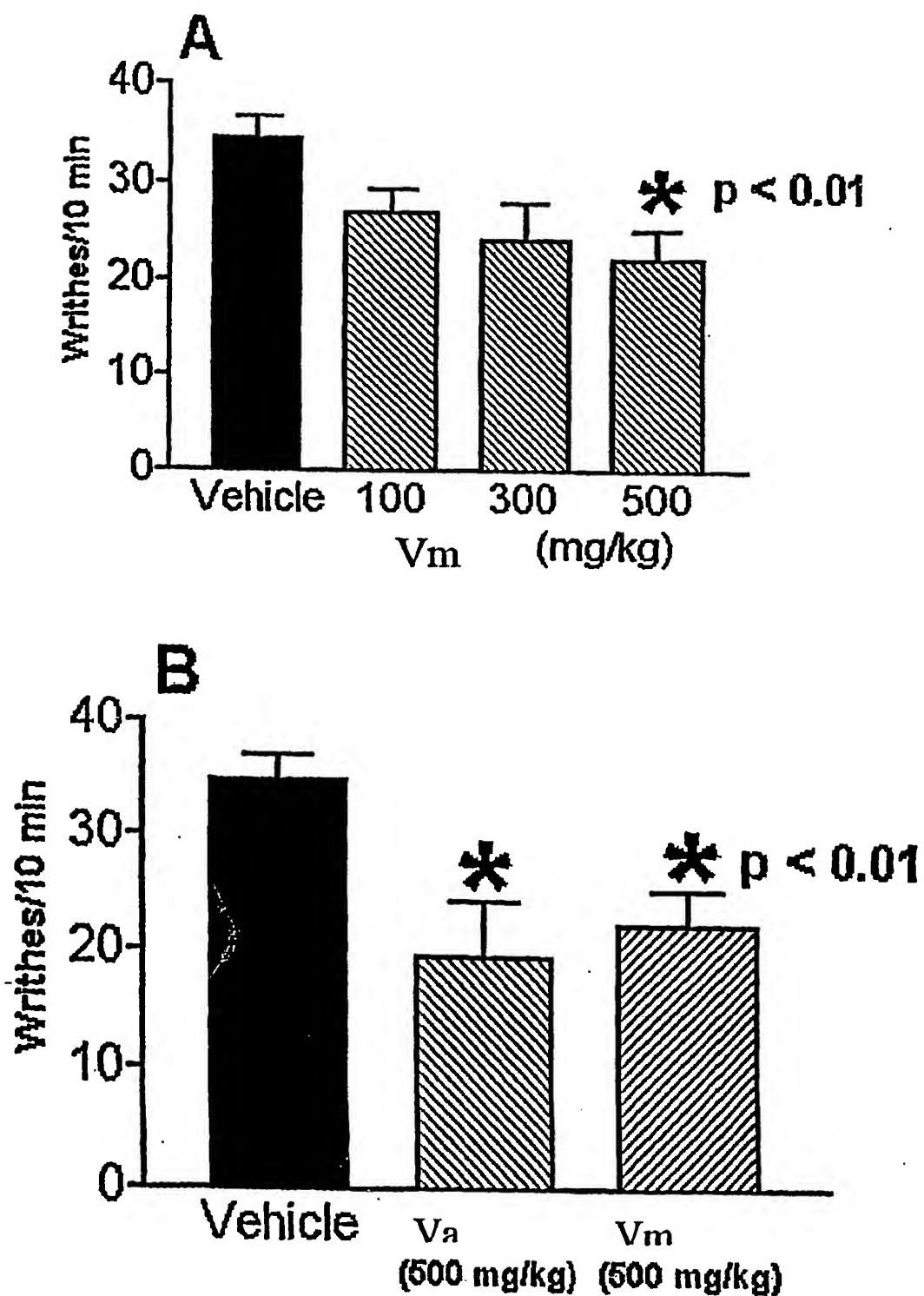
12/15

FIGURE 12



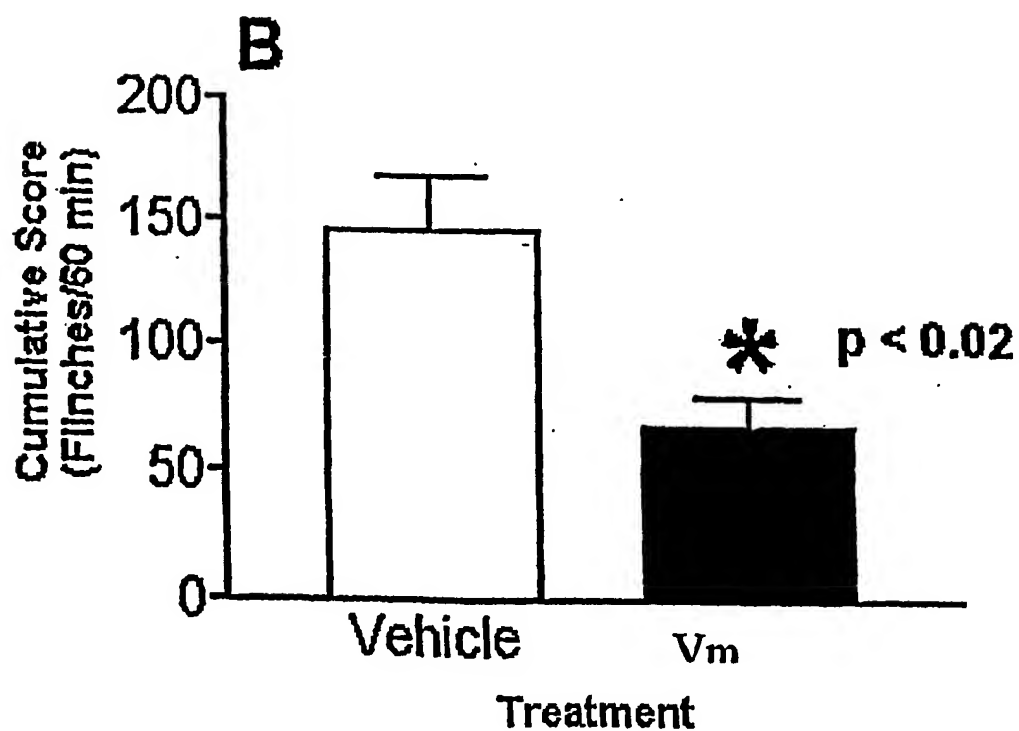
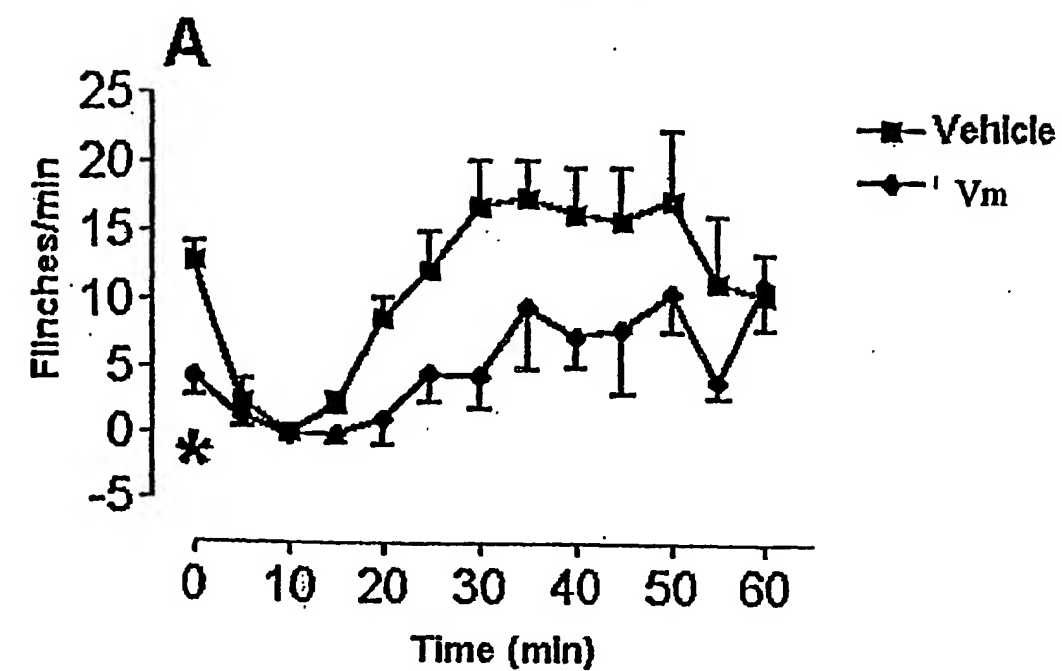
13/15

FIGURE 13

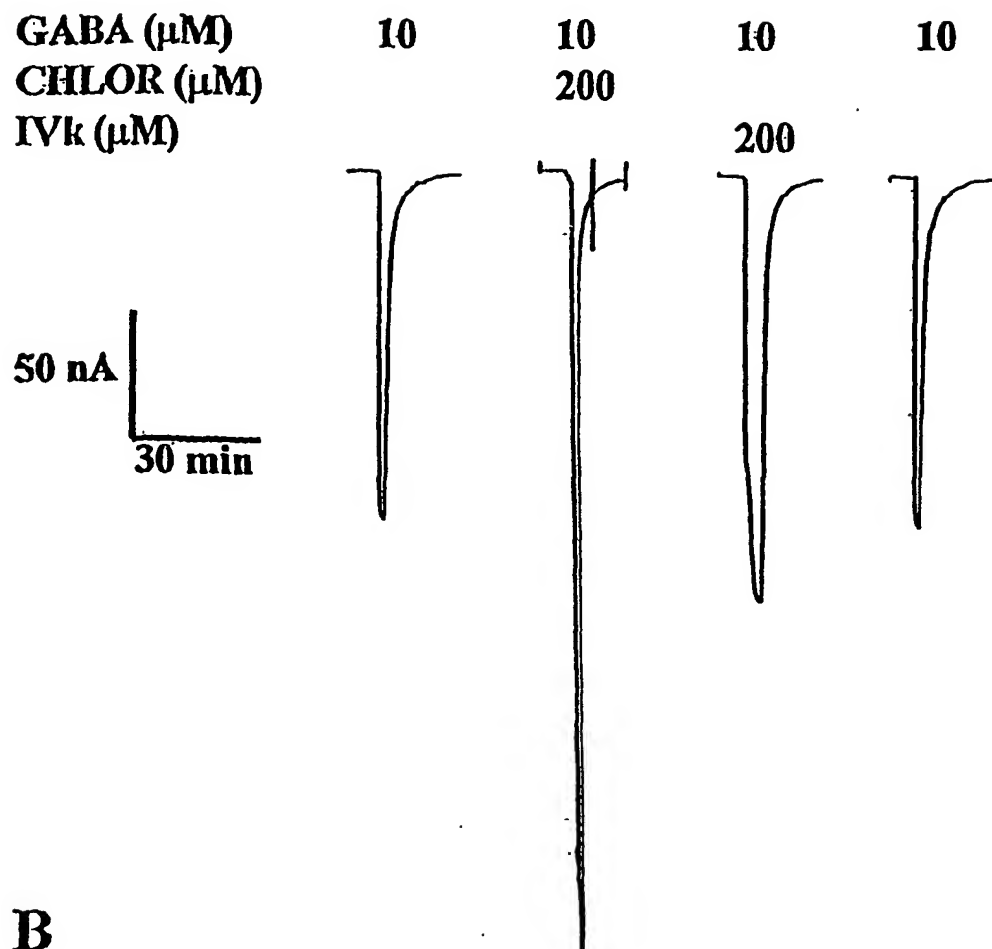


14/15

FIGURE 14



15/15

A**FIGURE 15****B**